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STUDIES IN THE ANATOMY OF SUGARCANE STALK

Part IV. Sampling Technique for the Evaluation of Anatomical Structure for Comparative Study

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I. Introduction

While reviewing literature in a recent paper (Khanna and Sharma, 1947), the authors found that very little work had been done on the economically useful portion, the stem, of sugarcane plant which was an important crop in more than a dozen countries. Earlier workers (Bremekamp, 1914, and Arstchwager, 1925) were mainly interested in the general structure of the stalk, and therefore, selected a portion of it, one of the top internodes, which was the easiest to section. Arstchwager (1925) used an internode taken from the upper third of a large cane as representative material for such a study. Ueno (1938) in his comparative studies took three successive internodes from top, middle and bottom portions of a stalk but there was no indication as regards the number and the method of selection of stalks whereas Ekambaram (1939) while studying the anatomy of sugarcane varieties and its hybrids with Sorghum, found the bottommost internode just above the ground level as most suitable for this type of investigation but he also did not adduce any data in support of his findings. Khanna and Panje (1939), however, mentioned having used internodes of three stalks, but they took from each stalk only two internodes, namely, the basal (the internode just above the one at ground level) and dry-leaf (the one having the uppermost dry leaf at the time of collection). Buzacott (1940) and Rao (1941) again did not refer precisely as to which portion of cane stalk was used in correlating the rind hardness with its anatomical structure.

In addition to the different number of talks and internodes taken from different portions of a stalk, no attempt was made by these workers except Khanna and Panje (*loc. cit.*) to give a precise mathematical expression to anatomical structure. The correlation between this character, the magnitude of which was estimated on visual observation and rind-hardness determined much more accurately by an instrument, was, therefore, based not on surer grounds of statistical scrutiny. Khanna and Panje attempted to define anatomical make-up of a stalk and were able to bring out the relation-

ship in a more tangible form by means of averages and graphs, but the data collected by them were not adequate for critical examination.

The anatomical structure of sugarcane stalk is typically monocotyledonous except that a large number of vascular bundles of various sizes are concentrated to form a comparatively hard peripheral zone, the rind—where both the vascular bundles and the parenchymatous matrix undergo rapid changes as one proceeds inwards from the epidermis. In the central region which begins at 1·5 mm. to 2·0 mm. from the epidermis, the tissues are more or less uniform throughout.

The evaluation of anatomical structure, therefore, resolves itself into the determination of the following features in these regions.

1. The area of vascular bundles in unit area of ground tissue;
2. The thickness of cell walls in parenchymatous and sclerenchymatous tissues;

and 3. The size of parenchymatous cells forming the ground tissue.

The present contribution gives a procedure to regularise the selection of material for comparative study and to estimate the magnitude of various anatomical features. Precision and time required for collecting data sufficient for statistical analysis with the facilities of an ordinary laboratory have been the main consideration in arriving at the procedure.

II. Enunciation of the Problem

In order to standardise the method of sampling and of the study of the three anatomical features, the whole process is resolved into seven successive steps given below at each of which an unknown factor is likely to creep in and thus vitiate the data and the conclusions drawn from them.

(1) How? and

(2) How many stalks should be selected?

(3) From which of the three regions of a stalk, namely, top, middle and bottom, an internode should be taken?

(4) Which portion of the selected internode should be sectioned?

(5) If the internode is too thick or too hard to cut so as to get an entire cross-section, which sector of the latter should be studied?

(6) How should the areas for the study be fixed?

and (7) For feature No. 1 (*a*) how many and (*b*) how should vascular bundles be measured for their size so that total area under them in a unit area of ground tissue may be found out, and for features Nos. 2 and 3, how many observations per sector should be taken?

On theoretical consideration, it will appear that in sampling for feature No. 1, the vascular bundle is much more variable than the other two elements coming into the picture, namely, the stalk and the internode and therefore the whole procedure is developed from below upwards, in other words from the smallest and the most variable element (the vascular bundles) to the largest and the least variable one (the stalk) involved in the study.

Each step has been dealt with separately as regards the material and methods used and the conclusions arrived at. For the thickness of cell walls and the number of parenchymatous cells per unit area, the procedure would naturally have to be modified to suit their requirements.

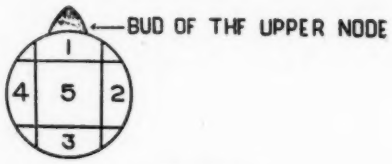
III. Vascular Bundles

(i) *Measurement of the Size.*—Since it is the vascular bundle which is to a very great extent, responsible for the mechanical strength and the fibre content of the stalk, the paramount importance of the accurate determination of its size need no emphasis.

Vascular bundles in the internodal region of sugarcane stalk are as regards their shape, essentially quadrilateral with zygomorphic symmetry along the plane passing through the protoxylem and protophloem. In the central portion of its cross-section, they are rhomboidal while in the periphery, due to the greater development of sclerenchyma at the xylem-pole to discharge one of the functions more effectively, they are elongated along their axes of symmetry which almost always coincide with the radii of a transverse section, and are therefore termed as radial. The axes usually at right angles to them and passing through both the metaxylem vessels,—the plane of maximum width,—are called the tangential. Parenthetically, although in the case of vascular bundles of the central region where they are scattered irregularly, these terms lose their original significance, yet they are retained for the sake of convenience. Vascular bundles nearest the epidermis which are the smallest of the lot are either flattened (tangential axis > the radial) or circular.

It would therefore appear that for a comparative study of the size of vascular bundles at different depths form the epidermis, the product of the two axes would give a very reliable index for all of them except a few of the smallest circular ones occurring in the peripheral region. To test this hypothesis, the size of all vascular bundles found in a circular unit area of ground tissue (1·9 mm. in diameter) in each of the four peripheral sectors (Text-Fig. 1) of upper half of middle internodes of three representative stalks was determined for three varieties of chewing canes, *viz.*, Saharanpur, Amritsar and Peshawar Paundas by the following three methods, a passing

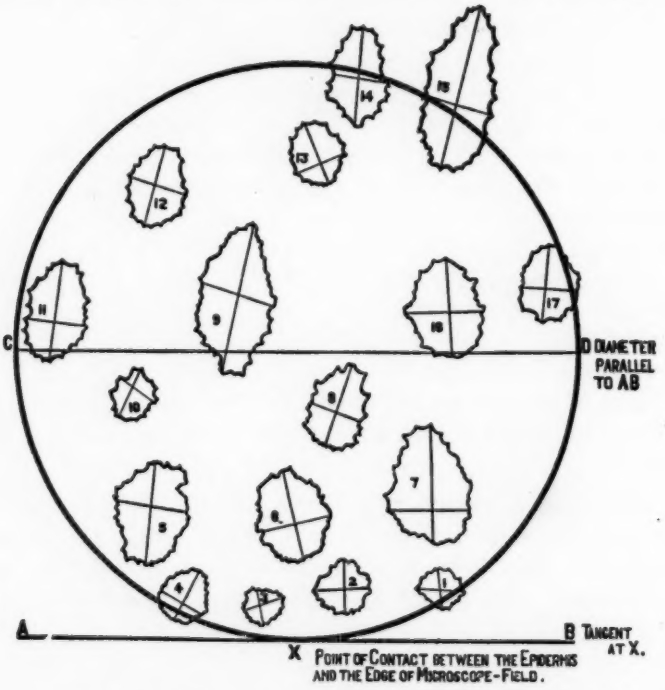
reference to which was made by the authors eleswhere (Khanna and Sharma, 1947).



TEXT-FIG. 1.

Method C.—The product of radial and tangential axes measured under microscope in terms of the divisions of an eye-piece micrometer (1 division, $= 17 \cdot 3\,\mu$) gave a comparative value of the size (Plate I).

Method B.—Those very vascular bundles were drawn 140 times magnified with the help of a camera lucida. The actual area of each figure was measured with a planimeter to the nearest 0·25 sq. cm. (Text-Fig. 2).



Unit Area of Ground Tissue showing 17 Vascular Bundles.

TEXT-FIG. 2. Camera lucida drawing of Plate I. ×140.

Method A.—The product of the two longest axes of the figure at right angles to each other, measured to the nearest mm. was taken as an index of the size of the figure, analogous to that of a vascular bundle found out by Method C (Text-Fig. 2).

These three sets of data for one unit area are given in Table I.

TABLE I

Showing the area of vascular bundles in unit area of ground tissue by the three methods

(Peshawar Paunda: Cane I, Sector I)

S. No. of vascular bundle	Method A			Method B	Method C		
	1st axis	2nd axis	Area	Area	Radial axis	Tangential axis	Area
	cm.	cm.	sq. cm.	sq. cm.	17·3 μ	17·3 μ	$17{\cdot}3^2$ sq. μ
1	1·8	2·8	5·04	2·75	9	8	72
2	2·5	2·5	6·25	4·25	10	10	100
3	1·5	2·1	3·15	2·00	8	7	56
4	2·4	2·4	5·76	3·75	11	10	110
5	4·7	3·2	15·04	11·00	16	15	240
6	4·1	3·5	14·35	10·00	20	16	320
7	5·1	3·8	19·38	14·25	20	15	300
8	3·8	2·5	9·50	7·25	16	10	160
9	6·7	3·4	22·78	16·00	28	14	392
10	2·5	2·0	5·00	3·25	9	8	72
11	4·7	3·0	14·10	9·50	18	13	234
12	3·8	2·9	11·02	8·00	15	13	195
13	3·0	2·5	7·50	5·50	12	9	108
14	4·2	2·6	10·92	8·50	16	11	176
15	7·5	3·1	23·25	17·50	29	12	348
16	4·3	3·7	16·91	10·75	18	13	234
17	3·7	2·6	9·62	6·00	11	9	99

Obviously the actual area of the figure (Method B) bore the most accurate relationship to the actual size of the respective vascular bundle. Method B was, therefore, the standard and correlation coefficients between Methods B and A and between B and C which were calculated separately for all the three varieties and pooled together, are given in Table II.

It would appear that correlation coefficient between Methods B and A and between B and C for each variety separately and for all varieties taken together were very high, more so in the former pair which was but natural, approaching almost unity, so clearly shown by the scatter of 517 points (Text-Figs. 3 and 4). The standard errors of the regression coefficients also were found to be low for both the pairs, for B–A pair as expected being

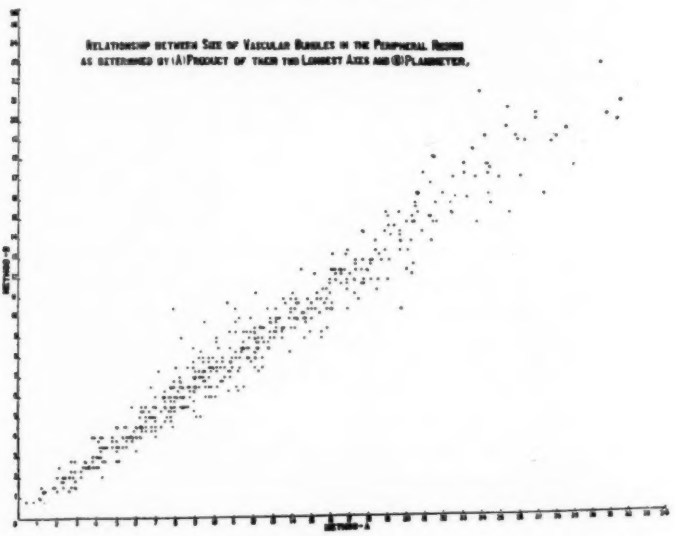
maller than that for B–C pair. The area of the figure could, therefore, be estimated accurately from both the products with the help of a linear equation. In other words, the size of a vascular bundle as determined by either of the

TABLE II

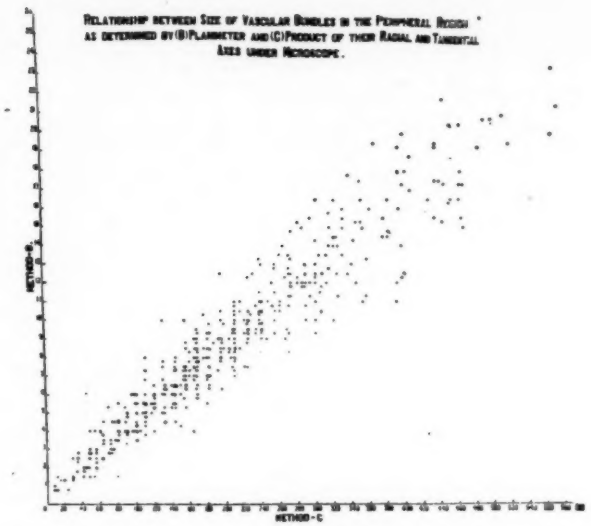
Showing correlation coefficients between the size as determined by three methods

Variety	No. of vascular bundles measured	Between B and A		Between B and C	
		Correlation coefficient	Regression equation	Correlation coefficient	Regression equation
Saharanpur Paunda	164	+0·9831 ±0·0144	Y=0·07+0·7026X ±0·0104	+0·9495 ±0·0247	Y=1·02+0·0382X ±0·0010
Amritsar Paunda ..	193	+0·9914 ±0·0095	Y=0·31+0·7244X ±0·0069	+0·9563 ±0·0220	Y=0·90+0·0392X ±0·0009
Peshawar Paunda ..	160	+0·9348 ±0·0282	Y=0·81+0·6359X ±0·0192	+0·9578 ±0·0229	Y=0·85+0·0390X ±0·0009
Varieties taken together	517	+0·9699 ±0·0107	Y=0·41+0·6868X ±0·0076	+0·9546 ±0·0133	Y=0·93+0·0388X ±0·0005

where X = A or C and Y = B.

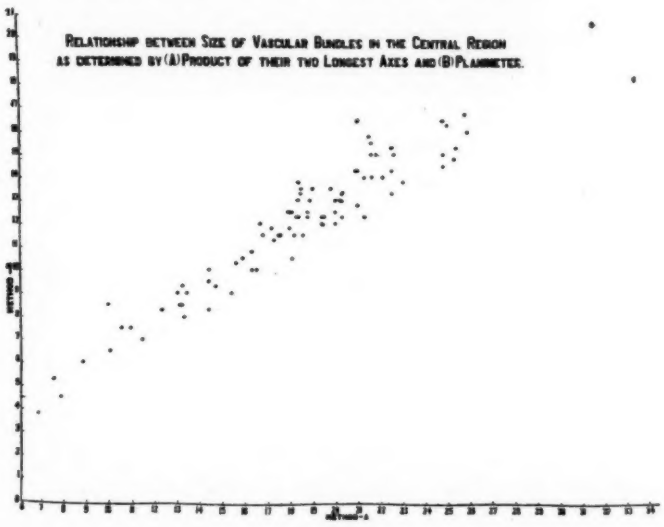

RELATIONSHIP BETWEEN SIZE OF VASCULAR BUNDLES IN THE PERIPHERAL REGION
AS DETERMINED BY (A) PRODUCT OF THEIR TWO LONGEST AXES AND (B) PLANIMETER.

TEXT-FIG. 3.


RELATIONSHIP BETWEEN SIZE OF VASCULAR BUNDLES IN THE PERIPHERAL REGION
AS DETERMINED BY (B) PLANIMETER AND (C) PRODUCT OF THEIR RADIAL AND TANGENTIAL
AXES UNDER MICROSCOPE.
METHOD-B
METHOD-C

TEXT-FIG. 4.
The scatter of 517 points in Fig. 3 is less diffuse than that shown in Fig. 4.

Methods A and C was a very highly reliable index of its actual size and could be used for all practical purposes in a comparative study.


RELATIONSHIP BETWEEN SIZE OF VASCULAR BUNDLES IN THE CENTRAL REGION
AS DETERMINED BY (A) PRODUCT OF THEIR TWO LONGEST AXES AND (B) PLANIMETER.
METHOD-B
METHOD-A

TEXT-FIG. 5. All the points are situated more or less within a narrow ellipse.

In the central region where the difference in the size and shape of vascular bundles was not so much as in the periphery, correlation between the size of 90 vascular bundles taken at random from the 5th sector, 10 from each as determined by Methods B and A, was found to be highly positive being of the order of $0{\cdot}8889 \pm 0{\cdot}0488$ with the corresponding regression equation being $Y = 1{\cdot}83 + 0{\cdot}5580\,X \pm 0{\cdot}0305$, where X = the size by Method A and Y = the size by Method B. In view of the close correspondence between this value and that for B and C in respect of vascular bundles occurring in the peripheral region, the latter was not calculated for central region, nor was it thought necessary to do so for each variety separately.

Of the Methods A and C, both of which were found to predict, with a high degree of precision, the size of vascular bundles, the former was slightly more accurate than the latter by virtue of its higher affinity with the ideal method, *i.e.*, B. But by Method C, a sufficiently large number of observations could be taken easily and quickly so as to provide data for statistical scrutiny which would more than compensate for any slight loss in accuracy. Method A was slow and laborious entailing the drawing of figures with the help of a camera lucida, the use of which was limited to some extent by the intensity of diffused light. Naturally the number of vascular bundles measured for their size would be small and the resultant data too meagre for critical examination. Drawing of figures takes so much time that after having drawn them, one would rather like to use planimeter than to resort to Method A. Therefore Method C being quick and almost equally as accurate as Method A is recommended.

(ii) *Number of Vascular Bundles.*—As the vascular bundles are scattered irregularly all over the field of a microscope which can conveniently be taken as unit area of the ground tissue, it is not possible to select, for the measurement of their size, the requisite number without any bias, unless all of them are numbered with the help of a camera lucida or an epidiascope, and then randomised. In view of this inherent difficulty and because of the wide variation in the size of vascular bundles occurring in rind, it was found convenient to measure all of them in unit area of that portion of the cross-section. Summation of the size of all the vascular bundles would give for that unit area of ground tissue, the total area under them, which it is easier to compare in different varieties than the size and number taken separately. These two characters were found to vary inversely with respect to each other but the correlation coefficient between them was not of the same order in different varieties (*vide* Appendix). More often it so happens that a variety has greater number of vascular bundles but of smaller size than the other and it is not possible to adjudicate one of them to be superior to the other

without in any way considering the size and number together. The comparison of various varieties is therefore shifted to a higher level, *viz.*, the total area under vascular bundles which is a function of both the attributes, the size and number, and includes these two characters in their entirety.

In the central region where the variability in size is rather low, 50–60% (5–6 vascular bundles in a circular unit area of 4·1 mm. diameter) of the total population of a unit area would give a very accurate estimate of their average size, which, when multiplied by their number, would be the total area under them.

(iii) *Unit Area of Ground Tissue.*—In order that the total area of vascular bundles is determined in portions of rind comparable to one another, the edge of the microscope-field should just touch the epidermis (Plate I, Text Fig. 2). It is also necessary that one of the vascular bundles touches or is very near an extremity of the diameter CD parallel to the tangent AB at the point of contact X in the epidermis. When more than two unit areas are to be taken, they should be contiguous, so as to guard against the same area or part of it being observed over again. Adjustment for part of a vascular bundle or bundles will have to be made judiciously.

(iv) *Sectors.*—Entire transverse sections of an internode, unless very young, are not easy to obtain by hand because of its girth or the hardness of the tissues. Microtoming by paraffin method is out of question while sections of fresh material taken with the help of a sledge microtome are too thick for other characters such as the thickness of cell walls. It is, therefore, always necessary to split an internode for cutting sections.

In view of the large area of a transverse section as compared to the area observed, the question naturally arises whether total area under vascular bundles per unit area of ground tissue, is the same in comparable regions of a cross-section, and if not, which of the sectors or a combination of them is the most representative of the whole, so that sectioning work may be reduced to the minimum.

Data on vascular bundles which were collected sector-wise, as already stated under III (i) were subjected to further statistical examination by taking canes, sectors and vascular bundles as successive sub-strata or zones and analysing them with help of the formula for stratified random sampling (Hierarichcal sampling) so that variances due to different sources could be estimated. As the material was collected on the basis of random selection as far as practicable, it is expected that the variances thus estimated would be near about the mark for all practical purposes. Analysis of variance of all

the three sets of data for the three varieties was done separately and for six other varieties collected by Method C (Table III).

TABLE III

Showing the estimate of variances due to different zones

Varieties	Method	Mean and S.E.	σm as % of mean	$\sigma 1^2$	$\sigma 1^2$ as % of total	$\sigma 2^2$	$\sigma 2^2$ as % of total	$\sigma 3^2$	$\sigma 3^2$ as % of total
1. Saharanpur	A	11·91± 0·7891	6·36	0·94	2·06	1·78	3·89	42·96	94·05
Paunda	B	8·53± 0·5798	6·80	0·57	2·72	1·13	5·42	19·19	91·86
	C	196·58±17·7800	9·04	671·92	4·91	155·80	1·14	12851·0	93·95
2. Amritsar	A	11·30± 0·4746	4·20	0·00	0·00	0·19	0·46	42·19	99·54
Paunda	B	8·50± 0·3856	4·54	0·00	0·00	0 38	1·69	22·35	98·31
	C	193·86± 9·4400	4·87	38·82	0·28	0·00	0·00	13620·0	99·72
3. Peshawar	A	12·64± 0·5274	4·17	0·00	0·00	0·00	0·00	44·50	100·00
Paunda	B	8·85± 0·3593	4·06	0·00	0·00	0·00	0·00	20·65	100·00
	C	205·04± 8·7400	4·26	0·00	0·00	0·00	0·00	12155·0	100·00
4. Co 213 ..	C	371·00±18·31	4·94	0·00	0·00	0·00	0·00	44·587	100·00
5. Co 313 ..	C	325·72±15·88	4·88	0·00	0·00	886·96	3·44	25·498	96·56
6. Co 356 ..	C	399·75±18·50	4·63	0·00	0·00	0·00	0·00	55·797	100·00
7. Co 395 ..	C	346·57±13·84	3·99	0·00	0·00	0·00	0·00	27·967	100·00
8. Co 453 ..	C	340·81±15·54	4·56	189·01	0·60	0·00	0·00	31·206	99·40
9. Co 513 ..	C	309·67±22·24	7·18	1113·90	5·33	0·00	0·00	19·768	94·67

where $\sigma 1^2$ = variance due to stalks.
$\sigma 2^2$ = do. sectors.
and $\sigma 3^2$ = do. vascular bundles.

Note.—If the mean square due to a particular hierarchy happens to be less than that in the immediately next one, the variation due to that hierarchy has been taken to be zero.

From the table given above, it would appear that the variance due to sectors was 0·00% of the total variance in the case of Peshawar Paunda and in Amritsar Paunda it did not exceed 1·69% even by the most accurate Method B. In the Saharanpur Paunda it was, however, found to be 1·14% by Method C and 5·42% by Method B. In two more sets of three varieties each, namely Co 213, Co 313 and Co 356 and Co 395, Co 453 and Co 513 studied by Method C alone, the sectors contributed 3·44% of the total variance in Co 313 while in the other five varieties, their contribution was 0·00%. Variations due to sector by the method recommended, namely C, was therefore almost negligible as it was not greater than 3·44% in any of the nine varieties studied by this method, it being 0·00% in seven of them. In other words, each sector was as representative of a cane cross-section as any of the other three.

The conclusion arrived at, in the last para was further substantiated when the total area under vascular bundles per unit area of ground tissue in the four sectors was taken into consideration separately or in various

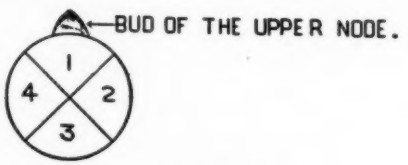
combinations as given in Table IV which was compiled by taking averages of the three readings for a sector.

TABLE IV

Showing the average area of vascular bundles per unit area of ground tissue in the four sectors of their different combinations

Sectors or their combinations	Area by Method A			Area by Method B			Area by Method C		
	Saharanpur Paunda	Amritsar Paunda	Peshawar Paunda	Saharanpur Paunda	Amritsar Paunda	Peshawar Paunda	Saharanpur Paunda	Amritsar Paunda	Peshawar Paunda
1 ..	160·67	183·30	179·00	115·07	137·73	127·63	2660·00	3225·67	2867·00
2 ..	154·37	183·43	156·73	109·53	139·90	110·43	2721·00	3202·00	2556·33
3 ..	162·43	177·93	169·00	117·33	132·73	118·80	2635·00	3070·33	2656·33
4 ..	173·87	184·30	169·13	124·40	136·20	115·10	2730·67	3136·33	2787·70
1+2 ..	157·52	183·37	167·87	112·30	138·82	119·03	2690·50	3216·20	2711·67
1+3 ..	161·55	180·63	174·00	116·20	135·23	123·22	2587·83	3148·00	2761·67
1+4 ..	168·73	183·80	174·07	119·73	136·97	121·37	2695·33	3181·00	2827·33
2+3 ..	158·40	181·68	162·87	113·42	136·32	114·62	2078·00	3123·00	2606·36
2+4 ..	164·12	183·87	162·93	116·97	138·05	112·77	2741·83	3162·60	2720·33
3+4 ..	168·15	180·12	169·07	120·87	134·47	116·95	2682·83	3103·33	2722·00
1+2+3 ..	159·16	181·56	168·24	113·98	136·79	118·96	2672·00	3161·50	2693·22
1+3+4 ..	165·66	181·84	171·27	118·93	135·56	120·51	2703·90	3186·25	2737·00
2+3+4 ..	163·56	181·89	164·96	117·09	136·28	114·78	2675·22	3144·11	2770·33
1+2+4 ..	162·86	183·68	168·29	116·33	137·94	117·72	2695·56	3128·00	2666·78
Grand Average (1+2+3+4)	162·83	182·24	168·47	116·58	136·64	117·99	2687·00	3155·00	2717·00

The averages of sectors separately or in different combinations did not differ appreciably from the average for the variety in all the three methods. Further, these small differences did not show any consistent trend of variation in favour of any particular sector or combination to merit further critical examination. Theoretically therefore, any sector can be taken. Still it is always better to have a fixed sector. Because of the distinctive feature of having the bud at the upper node, Sector 1 or quadrant 1 (Text-Fig. 6) where the girth of the internode is not thick enough to be divisible in to five sectors, is fixed for the purpose.



TEXT-FIG. 6.

It is interesting to note the similarity in the trend of variations due to various sub-strata (Table III) in the data collected by three different methods.

The percentages shown by these methods in different zones of a variety are of the same order which further supports the conclusions arrived at under III (i).

(v) *Portion of the Internode.*—The internal structure of a stalk from leaf-scar to leaf-scar varies so much that sections takes a few millimeters apart in certain portions of the internodal length, do not bear any resemblance to one another. Bloom band, root zone and growth ring are highly unstable from that point of view and are therefore not at all suitable for comparative study.

Internode proper (the portion lying between the growth ring and the bloom band) is the most stable except in its lowermost portion where transition in the structure and orientation of vascular bundles, typical of the growth ring to that of the internodal region takes place. Ueno (1938) found that rind-hardness was more or less the same between 6 and 12 cm. from the lower leaf scar in an internode of P.O.J. 2725 (nearly 15 cm. in length) in all the three regions of a stalk. Therefore to steer clear of all the structural changes which take place in the lower portion of an internode proper for a distance of nearly 3–4 cm. from the growth ring, it is suggested to take the upper half of the total length (from leaf scar to leaf scar) of an internode. This has the further merit of a section being easily distinguishable from the normal one, if it happens to pass through the bloom band where vascular bundles undergo great structural changes and are surrounded by readily elongated parenchymatous cells arranged stellate fashion. These modifications are so striking and in such clear contrast to the normal structure that it is much easier to say definitely whether or not a section has been taken from the stable portion in the case of the upper half of an internode than in that of the lower where structural changes consist of slow replacement of collenchyma by sclerenchyma and gradual reduction in the size of vascular bundles.

(vi) *Region of Stalk.*—The question as to the selection of proper internode out of nearly 15–20 internodes which a stalk is usually made of, and which by virtue of their being formed in succession at different periods of growth, are at various stages of development, was tackled by dividing, roughly on length basis, a stalk into three pieces, bottom, middle and top and taking the middle internode of each piece for sectioning.

Statistical analysis of average area under vascular bundles in five unit areas of matrix in the peripheral region of these internodes taken from three stalks for each of the six varieties, namely Co 205, Co 213, Co 214, Co 290, Co 331 and *Saraitha* (Table V) showed that 16 out of 18 bottom internodes

TABLE V *Showing average total area under vascular bundles in the peripheral and central regions of bottom, middle and top internodes of three stalks of six sugarcane varieties*

Varieties	Region	Stalk No. 1				Stalk No. 2				Stalk No. 3				No. of stalks without sig. diff. between		
		Bottom	Middle	Top	C.D. at 5% level	Bottom	Middle	Top	C.D. at 5% level	Bottom	Middle	Top	C.D. at 5% level	Bottom and middle internode	Bottom and top internode	Middle and top internode
Co 205	Peripheral	3897·6	3774·8	2956·6	405·16	3578·4	3821·2	2622·6	463·50	3666·6	3848·6	2505·2	453·21	3	0	0
	Central	1370·8	1388·6	1194·4	456·09	1776·8	1517·8	1082·2	472·15	1294·0	1259·6	1063·8	468·11	3	2	3
Co 213	Peripheral	2167·6	2343·4	1684·6	390·96	2323·4	2084·2	2060·6	401·35	2740·0	2354·4	2018·0	240		1	1
	Central	1030·0	1207·8	736·0	227·90	1179·6	893·6	903·2	435·91	1130·8	916·4	843·6	257·17	3	2	2
Co 214	Peripheral	2948·4	2698·8	2703·8	294·03	3479·6	2802·0	2818·8	339·79	3070·4	3183·6	3262·3	368·16	2	2	3
	Central	1398·4	1193·4	1559·2	327·83	1537·0	1257·8	1406·2	362·59	1584·0	1399·6	1720·8	512·41	3	3	2
Co 290	Peripheral	2859·4	2892·4	3314·0	549·63	3190·4	3430·0	2942·0	609·88	3409·8	3243.6	2918·8	694·86	3	3	3
	Central	1320·8	933·6	1438·4	455·02	1124·2	921·2	1378·0	488·20	1128·4	977·8	1218·8	460·66	3	3	2
Co 331	Peripheral	3521·8	3654·2	2668·6	518·97	3021·4	3326·8	2561·2	648·03	3655·6	3598·2	2842·8	628·75	3	1	0
	Central	1663·8	952·8	884·6	463·12	1429·4	1486·0	933·0	415·06	1079·6	1413·2	1153·0	505·68	2	1	2
Saraitha	Peripheral	2898·6	2660·2	..	288·27	2652·6	2885·6	..	274·07	2616·6	2892·4	..	443·24	3	..	..
	Central	1395·4	1192·6	..	345·07	1332·8	1411·0	..	269·02	1088·8	1354·2	..	142·00	2	..	..

were not significantly different from their corresponding middle internodes even at 5% level, and that both of them did not differ significantly from the top internode in seven canes. Similarly in the central region the difference between the bottom and the corresponding middle internode was not significant in 16 out of 18 stalks. Top internodes in central region were found to differ significantly from the corresponding bottom and middle internodes in eleven stalks.

Therefore so far as this character was concerned, bottom and middle internodes represented, in equal measure, the stalk from which they were taken. That a top internode was definitely out of the picture as a representative of a cane, was evident from the fact that in the peripheral region in seven out of fifteen stalks, it was significantly different from both bottom and middle internodes and in another two from either of them; only in six canes there were no significant differences between top and middle and between top and bottom internodes. When central region was taken into consideration, the number of stalks without significant differences amongst these three internodes, rose to nine; in two of the remaining six stalks, top internode was totally different from both bottom and middle internodes, and in four from either of them.

The conclusions arrived at in the preceding paragraphs were supported by the observations on rind hardness by Ueno (*loc. cit.*). He found that except in the growth-ring segment, the difference in the rind-hardness of the other three segments of the second internodes of middle and bottom regions was nearly 25–33% of that prevailing between those of the top and middle regions. The rind hardness in the three segments, namely root zone, internode (proper) and bloom band, of the lowest internode of the middle region, was more or less the same as that of the uppermost internode of the bottom region.

Although either of the bottom and middle internodes can be taken as an equally good representative sample of a stalk, yet in view of what has been stated in last sub-section, middle internode proves to be superior to the bottom one, because of its greater length. As is well known, a stalk earliest to emerge is, in its lower region, made up of internodes too short to be sectioned as stated before. It is only in the middle region that internodes are stabilized as regards their length. Even in a variety characterised by short internodes, in this region they are long enough to yield an upper half of adequate length for sectioning purposes.

(vii) *Number of Stalks.*—A sample for anatomical study in the case of a sugarcane stalk comprises of three components, namely vascular bundle,

sector and stalk, all of which contribute their share to the total variance. The variance due to a particular component when expressed as percentage of the total variance indicates its importance in the sample in respect to the other two. A reference to Table III which was compiled on the basis of data collected from three stalks of each variety shows that the variance due to stalks was 0·00% of the total variance found in the sample in five out of nine varieties. In another two it did not exceed 0·60%. Only in the case of the Saharanpur Paunda and Co 513, it was respectively 4·91% and 5·33%.

Also the initial sample of three stalks of each of the seven out of nine varieties enlisted in Table III, gave a standard error of the mean which when expressed as percentage of the latter did not exceed 5%. Taking this (5%) as margin of error, the size of sample required for accurate estimation of the size of vascular bundles and consequently the area under them per unit area of parenchymatous ground tissue, would be more or less the same as the initial one in these seven varieties. For the other two, the required sample would have to be enlarged by taking more stalks. In the case of the Saharanpur Paunda and Co 513, respectively 9 and 7 stalks would form the required sample.

The initial sample might therefore consist of 3 stalks which in a large number of varieties would also be the required sample. If on the analysis of data collected from them, it was found that variance due to stalk approached a value necessitating the examination of a large number of stalks, more of them might be sectioned and studied.

(viii) *Selection of Stalk.*—A sugarcane plant at harvest consists of a number of stalks which emerge at different periods (from March to September in Bihar) and are at various stages of development. The mother shoot, the first to be produced, is usually the tallest of all unless attacked by a pest or disease and is followed by daughter shoots of *b*, *c*, *d*, and sometimes of *e*-order. The stalks also differ in the number and length of internodes forming them. On one hand, the mother shoot consists largest number of internodes the basal ones of which are the smallest, progressively increasing in length till they acquire their normal length in the middle region, while on the other, the daughter shoots of higher order say *d*- or *e*-, formed late in August or early in September, emerge with an internode which in length is equal to or greater than the internodes of the middle region of mother shoot. It is, therefore, essential that internodes produced at the same period should be taken for comparative study. In other words, shoots of the same age should be selected.

Ordinarily a mother shoot is the best for the purpose. It has acquired the greatest degree of maturity and is the easiest to locate because of its

comparatively short internodes in the basal region. Where it has died, the earlier daughter shoots of *b*-order resemble it very much. Therefore to be definite on the point, stalks for anatomical study should be properly tagged at the time of emergence. It need not be stated that a sufficiently large number of them have to be labelled so as to allow for the deaths caused by attacks of pests and diseases.

(ix) *Conclusion.*—In view of what has been stated in the preceding subsections, a sample for the study of vascular bundles in sugarcane stalk will ordinarily consist of upper half of three middle internodes taken one each from mother shoots which have been tagged at the time of emergence. Measurement of the size of all the vascular bundles found in four unit areas of parenchymatous matrix in a transverse section of Sector No. 1 will give fairly accurate estimate of the total area under them in peripheral region. For very precise investigation, one unit area per sector may be taken. In view of much greater uniformity in the size and number of vascular bundles in unit area in the central region than that prevailing in the peripheral region, the size of the sample for this region will not be more than 12 unit areas, *i.e.*, 4 determinations per stalk. If on the analysis of these data, any component is found to have acquired undue importance, the sample may be enlarged in that direction.

IV. The Thickness of Cell-Walls

The thickness of cell-walls in both the tissues is different at different depths of a transverse section of a stalk which, as already stated, can roughly be divided into a peripheral region of high lignification, the rind, and central region where the thickening of cell-walls is comparatively poor. The difference in the thickness of cell-walls in the peripheral and central regions was much greater in the case of the sclerenchyma than in that of parenchyma. But in the latter tissue, the reduction in its magnitude was gradual and spread over greater part of the cross-section, whereas in the former, it was more or less abrupt once the size of vascular bundle was stabilized.

Therefore, measurement of this feature at three depths, *i.e.*,* 0·6 mm. and 1·2 mm. from the epidermis in the peripheral region and in central appeared to be necessary in the case of the parenchymatous ground tissue, while for sclerenchyma,† determination in two regions seemed to be sufficient for the purpose. Data were collected for six varieties, 120 readings

* It was convenient to measure the thickness of cell walls at these depths with the combinations used, the eye-piece being a Ramsden Screwmicrometer and the objectives 8 & 40 and also because the rind was about 1·5 mm. thick.

† The thickness was measured at its maximum in the sheaths of 2–3 biggest vascular bundles in the field fixed by the abovementioned combination.

each (10 observations per sector, 4 sectors and 3 stalks) taken at each depth for both the tissues and 30 readings for each tissue in the centre. For another three varieties, only 16 measurements (2 stalks × 8 readings) were done for each tissue in these two regions.

(i) *Peripheral Region*: (*a*) *Parenchyma.*—The thickness of cell wall in parenchyma was found to be much more variable than the size of vascular bundles as was clear from the range of standard error expressed as percentage of the mean. It was as low as 2·20% in Saharanpur Paunda as against 15·74 in Co. 313 (Table VI) while in the case of vascular bundles it varied

TABLE VI

Showing the estimated variance due to different zones in the thickness of cell walls in parenchymatous tissue

Varieties	At	Mean and S. E.	S. E. expressed as % of the mean	$\sigma 1^2$	$\sigma 1^2$ expressed as % of total variance	$\sigma 2^2$	$\sigma 2^2$ expressed as % of total variance	$\sigma 3^2$	$\sigma 3^2$ expressed as % of total variance
Co 213	0·6 mm.	12·62±0·83	6·58	1·51	21·85	1·89	27·35	3·51	50·80
	1·2 mm.	11·57±·066	5·70	1·08	20·77	0·52	10·00	3·60	69·23
Co 356	0·6 mm.	15·67±2·18	13·91	13·18	56·42	3·50	14·98	6·68	28·60
	1·2 mm.	14·68±2·31	15·74	14·91	58·63	3·39	13·33	7·13	28·04
Co 313	0·6 mm.	12·70±1·74	13·70	8·45	50·72	2·04	12·24	6·17	37·03
	1·2 mm.	11·90±1·28	10·76	4·16	50·30	1·64	19·83	2·47	29·87
Amritsar	0·6 mm.	9·72±0·06	3·81	0·00	0·00	1·39	37·37	2·33	62·63
Paunda	1·2 mm.	8·10±0·34	4·20	0·00	0·00	1·24	50·41	1·22	49·57
	In centre	7·17±0·29	4·04	0·11	7·05	..	..	1·45	92·95
Pesha-	0·6 mm.	8·81±0·40	4·54	0·37	11·94	0·15	4·84	2·58	83·33
war	1·2 mm.	8·15±0·22	2·74	0·03	1·36	0·29	13·12	1·89	85·52
Paunda	In centre	7·73±0·26	3·36	0·00	0·00	..	..	1·96	100·00
Saharan-	0·6 mm.	8·00±0·32	4·00	0·09	3·75	0·70	29·17	1·61	67·08
pur	1·2 mm.	6·81±0·15	2·20	0·00	0·00	0·15	11·81	1·12	88·19
Paunda	In centre	6·63±0·26	3·92	0·09	7·32	..	..	1·14	92·68
Co 395	0·6 mm	17·25±0·72	4·17	0·00	0·00	0·00	0·00	8·20	100·00
	1·2 mm.	15·75±0·99	6·29	0·00	0·00	..	..	15·77	100·00
	In centre	11·94±0·65	5·44	0·00	0·00	..	..	6·85	100·00
Co 453	0·6 mm.	19·00±1·75	9·21	4·17	21·05	..	..	15·64	78·95
	1·2 mm.	16·00±0·87	5·44	0·75	10·68	..	..	6·27	89·32
	In centre	10·00±0·38	3·80	0·00	0·00	..	..	2·27	100·00
Co 513	0·6 mm.	17·69±1·44	8·14	2·61	17·66	..	..	12·17	82·34
	1·2 mm.	13·81±1·31	9·49	2·05	15·46	..	..	11·21	84·54
	In centre	12·88±1·22	8·70	2·05	34·92	..	..	3·82	65·08

where $\sigma 1^2$ = Variance due to stalks.
$\sigma 2^2$ = do. sectors.
$\sigma 3^2$ = do. cell walls.

Note.—1. If the mean square due to a particular hierarchy happens to be less than that in the immediately next one, the variation due to that hierarchy has been taken to be zero.

2. Thickness of two adjoining cell walls expressed as divisions of eye-piece micrometer each of which was equal to 0·21 μ.

TABLE VII *Showing the size of required sample (the number of stalks and the corresponding number of measurements) for the determination of the thickness of cell-walls in the parenchymatous and sclerenchymatous tissues in the peripheral region within a margin of 5% error of the mean as estimated from an initia sample of* 120 *readings* (3 *stalks*, 4 *sectors*, 10 *readings per sector*)

Stalk No.	1			2			3			4			5			6			7			8			More than 8					
Tissues / Variety	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at		Sclerenchyma	Parenchyma at 0·6 mm.		Parenchyma at 1·2 mm.		Sclerenchyma	
	0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		0·6 mm.	1·2 mm.		Stalks	Readings	Stalks	Readings	Stalks	Readings
Co 213 ..			52			16					128	16		40		48			56	28		32								
Co 356 ..															120						56			32	25	200	25	600		
																									26	104	26	208		
																											28	112		
Co 313 ..																									22	2728	13	988	32	1024
																									23	368	14	112	33	264
																									24	192	15	60	35	140
																									26	104				
Amritsar Paunda ..				32	96		24	24		16	16																		14	168
																													15	120
																													16	64
Peshawar Paunda ..					16	32	48	12	12	32			20																	
Saharanpur Paunda		16		48	16		24	12					20																11	176
																													12	96
																													13	52
Co 395 ..	12	26	11	12	26	10	12	27	9																					
Co 453 ..			10		24	10		18	2		16		230			78			56											
Co 513 ..										100			50	170		36	84			63									41	328
																													42	294
																													45	135

Note.—For the last three varieties the initial sample consisted of 16 measurements only (2 stalks, 8 readings per stalk).

between 3·99% and 4·94% in seven out of the nine varieties and did not exceed 9·04% in the two exceptions.

Likewise the percentage of variance due to the three zones was widely different in different varieties. Thus stalk was responsible for 1·36% of the total variance in Peshawar Paunda while its quota was as high as 58·63% in Co 356. In the case of the vascular bundles, the maximum variance due to this zone was 5·33%, it being below 0·60% in seven varieties. Similarly variance due to the 2nd and 3rd zones, namely the sector and cell also was not of the same uniform order as in the vascular bundles.

As a result of this hectic zonal behaviour, the size of the required sample so as to estimate the magnitude of this feature at 0·6 mm. within a margin of 5% error, was 12 readings, *i.e.*, 3 readings per sector of a stalk in the case of Co 395 while in Co 313 it was 2728 observations (31 readings per sector, 4 sectors and 22 stalks) (Table VII). At 1·2 mm. the corresponding readings were 26 from one stalk and 988 (19 per sector, 4 sectors and 13 stalks) from the varieties mentioned above.

With a view to minimize work on this aspect, the difference between the means at 0·6 mm. and 1·2 mm. was tested as to its significance and it was found that in eight out of nine varieties, it was not significant even at 5% level, the exception being Saharanpur Paunda in which the two means were significantly different even at 1% level. It might, therefore, be stated that thickness of cell-wall at 1·2 mm. was equally representative of the peripheral region as a whole.

The size of the required sample at this depth was found to be 12 to 27 observations (taken from all the four sectors of three stalks) in five out of nine varieties. Of the remaining four, Co 313 and Co 356 were the most refractory and very wide off the mark, while Co 213 and Co 513 required, respectively 128 readings from 4 stalks and 170 readings from 5 stalks. It is, therefore, suggested that the initial sample for this feature should consist of 36 observations (3 stalks, 4 sectors and 3 readings per sector) at a depth of about 1 mm. from the epidermis.

(*b*) *Sclerenchyma.*—The observations regarding the variability of thickness of cell walls made above were applicable to sclerenchyma with much greater force. The standard was found to vary from 2·99% of the mean in Co 213 to 22·81% in Co 513 and the variance due to stalk rose to 74·87% in the latter variety whereas it was 0·00% in Co 453 (Table VIII). The sector—and cell—zones were no less erratic in their contribution to the total variance.

Note.—For the last three varieties the initial sample consisted of 16 measurements only (2 stalks, 8 readings per stalk).

TABLE VIII

Showing the estimated variance due to different zones in the thickness of cell-walls in the sclerenchymatous tissue

Varieties	Region	Mean and S.E.	S.E. expressed as % of the mean	$\sigma 1^2$	$\sigma 1^2$ expressed as % of total var.	$\sigma 2^2$	$\sigma 2^2$ expressed as % of total var.	$\sigma 3^2$	$\sigma 3^2$ expressed as % of total var.
Co 213 ..	Periphery	26·38± 0·79	2·99	0·14	0·65	5·14	23·80	16·32	75·56
Co 356 ..	,,	26·24± 1·65	6·29	2·59	6·23	20·62	49·62	18·35	44·15
Co 313 ..	,,	32·06± 5·22	16·28	73·39	56·16	30·94	23·60	26·46	20·25
Amritsar	,,	21·96± 2·33	10·61	7·01	13·43	36·44	69·82	8·74	16·75
Paunda	In centre	12·23± 0·50	3·78	0·31	6·42	..	..	4·52	93·58
Peshawar	Periphery	17·55± 0·67	3·82	1·05	12·64	0·48	5·78	6·78	81·59
Paunda	In centre	14·07± 0·19	1·35	0·60	11·98	..	..	4·41	88·02
Saharanpur	Periphery	18·21± 1·71	9·39	7·56	37·54	3·78	18·77	8·80	43·69
Paunda	In centre	14·97± 1·63	10·89	7·53	61·22	..	..	4·77	38·78
Co 395 ..	Periphery	70·94± 2·81	3·96	2·87	2·70	..	..	103·60	97·30
	In centre	23·56± 1·28	5·43	0·00	0·00	..	..	26·38	100·00
Co 453 ..	Periphery	63·56± 2·48	3·90	0·00	0·00	..	..	98·21	100·00
	In centre	15·50± 0·75	4·84	0·78	21·85	..	..	2·79	78·15
Co 513 ..	Periphery	52·81±12·06	22·84	279·29	74·87	..	..	93·74	25·13
	In centre	33·00±11·38	38·45	255·18	89·85	..	..	28·84	10·15

where $\sigma 1^2$ = Variance due to stalks.
$\sigma 2^2$ = do. sectors.
and $\sigma 3^2$ = do. cell walls.

Note.—1. If the mean square due to a particular hierarchy happens to be less than that in the immediately next one, the variation due to that hierarchy has been taken to be zero.

2. Thickness of two adjoining cell walls is expressed as divisions of eye-piece micrometer each of which was equal to 0.21 μ.

The sample-size suggested for the parenchyma was found to be adequate for this tissue also for four out of nine varieties. An enlarged sample of 120 readings (5 stalks, 4 sectors, 6 readings per sector) was in no way superior because it brought in only one more variety. The initial sample in this case also was fixed in the first instance at 3 stalks 36 readings in conformity with that for the other tissue and other anatomical features. The chances of its being the required sample also might roughly be stated to be 50–50.

(ii) *Central region.*—The thickness of cell-walls in both the tissues in this region was more or less as variable as in the peripheral region, because the standard errors expressed as percentage of their respective means were of the same order except for sclerenchyma in Amritsar Paunda (Tables VI and VIII). A sample consisting of 3 stalks and 8 readings per stalk was found

to be adequate for all the varieties except Co 513 and Saharanpur Paunda in respect of sclerenchyma (Table IX).

TABLE IX

Showing the size of required sample (the number of stalks and corresponding number of measurements) for the determination of thickness of cell-walls in central region within a margin of 5% error of the mean as estimated from an initial sample of 30 readings (3 stalks, 10 readings per stalk) for first three varieties and 16 readings (2 stalks, 8 readings per stalk) for the remaining three varieties

Stalks	1		2		3		4		5		6		More than 6 stalks			
Tissue	P	S	P	S	P	S	P	S	P	S	P	S	Parenchyma		Sclarenchyma	
Variety													No. of Stalks	No. of readings	No. of stalks	No. of readings
Amritsar Paunda	77	36	20	16	18	15										
Saharanpur Paunda	61	..	18	..	15	..									14	196
															15	90
															16	64
Peshawar Paunda	13	..	14	24	15	15	..	16								
Co 395 ..	20	19	20	20	21	21										
Co 453 ..	10	..	10	14	12	9	..	8								
Co 513 ..											60	..	7	35	95	855
													8	32	100	200

Note.—P = Parenchyma; S = Sclerenchyma.

(iii) *Other Relevant Precautions.*—Of the seven successive steps enumerated under Section II, four namely, No. 2, 5, 6 and 7, have been dealt with while fixing the size of a sample. As regards Nos. 1 and 4 what has been stated under vascular bundles, holds good for this feature as well. Thus there remains only the question of middle internode *versus* bottom internode.

For this feature, the method followed for vascular bundles, *i.e.*, comparison of area under them in unit area in middle internode with that in the bottom internode of the same stalk was not found feasible because the two internodes were not at the same stage of development, so far as this feature was concerned. In the case of vascular bundles, once a primordium is fully differentiated into a vascular bundle, there is no further change as regards its area in transverse section and the character is stabilized in all internodes as soon as they reach their normal length and girth. But the thickness of

cell-wall is continuously being added to by slow deposit of lignin and is always on the move, like the gradual change taking place in the quality of sugarcane-juice. It is therefore, obvious that the middle and bottom internodes are not comparable as regards this feature as they are not for juice quality.

Pursuing the analogy between the thickness of cell-wall and the quality of juice, it will appear that the bottom internode like the top one is not representative of the stalk as a whole for both the features, because they represent the two extreme stages of development. The middle internode by virtue of its being situated in between the two, is much nearer the mark than the other two. The thickness of cell-walls in this internode was found to bring out the varietal differences much more clearly than that of the bottom internode which represented not the average but the maximum lignification a variety was capable of. In the bottom internodes, the differences between varieties were narrowed down, more so in the case of sclerenchyma (Table X, Text-Fig. 7). The thickness of cell-walls in this tissue, when expressed

TABLE X

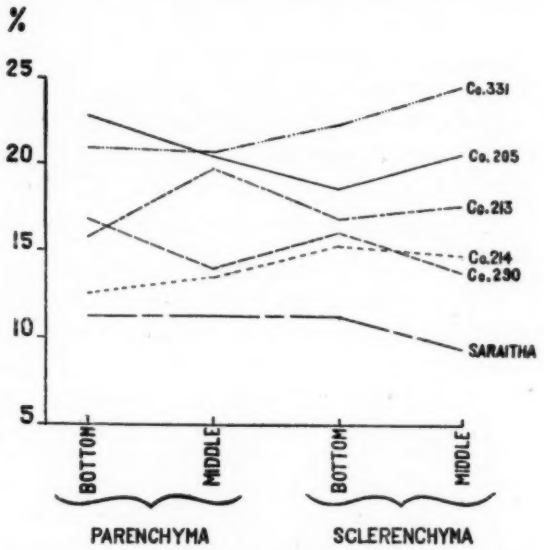
Showing the average thickness of cell-walls in peripheral region in bottom and middle internodes in six sugarcane varieties and as percentage of the total

Varitey	Thickness of cell wall				Percentage of the total			
	Parenchyma		Sclerenchyma		Parenchyma		Sclerenchyma	
	Bottom Internode	Middle Internode	Bottom Internode	Middle Internode	Bottom Internode	Middle Internode	Bottom Internode	Middle Internode
Co 205 ..	36·0	21·1	50·9	48·2	22·8	20·6	18·6	20·5
Co 213 ..	24·8	20·2	45·6	41·1	15·7	19·7	16·7	17·5
Co 214 ..	19·7	13·8	41·5	34·6	12·5	13·5	15·2	14·7
Co 290 ..	26·7	14·4	43·7	32·0	16·9	14·1	16·0	13·6
Co 331 ..	33·0	21·2	61·1	57·4	20·9	20·7	22·3	24·4
Saraitha ..	17·6	11·6	30·8	21·7	11·2	11·3	11·3	9·2
Total ..	157·8	102·3	273·8	235·0	..	..		..

Note.—Thickness of two adjoining cell-walls is given in terms of the divisions of eye-piece micrometer each of which was equal to 0·21 μ.

as percentage of the total, varied from 9·2% to 24·4% in middle internode while its range in the bottom internodes was 11·3% to 22·3%. A striking example where varietal differences were almost obliterated was provided by

Co 213 and Co 290 in which the thickness of cell-wall was more or less equal in both the tissues in the bottom internodes. That Co 213 was more lignified than Co 290, as was actually the case, was clearly brought out by



TEXT-FIG. 7. Thickness of cell walls in both the tissues in bottom and middle internodes of six varieties.

the thickness of cell-walls in the middle internodes which, therefore, depicted the true position in respect of this feature.

The middle internodes of tagged mother shoots of a variety or of different varieties were comparable, because having been formed at the same period, they were, of the same age. The lignification of tissues, which, like the quality of juice, was a varietal character, would also be at the same stage in different mother shoots of the same variety or at a stage comparable in different varieties. Although no experimental evidence was collected for the former feature, work carried on at the station on the latter, showed that there existed a constant relationship between the juice quality of the middle internode and that of the whole stalk (Khanna, 1938 and 1939). It might, however, be stated that the relative position of varieties in respect of the thickness of cell-walls in both the tissues as shown by the middle internode (Text-Fig. 7) was maintained in the bottom internodes as well, except that the differences inthe latter were narrowed down as already stated and that Co 290 was rather erratic when considered in respect of other varieties. Within the variety

itself lignification in middle and bottom internodes was however, more regular than in all other varieties.

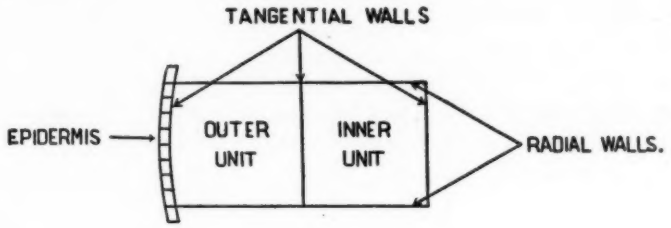
(iv) *Conclusion.*—The size of a sample for the thickness of cell-walls in peripheral region might, therefore, be suggested at 36 readings taken 3 each from all the four sectors of middle internode of 3 mother-shoots, and for the central region 24 measurements, *i.e.*, 8 per stalk. Such a sampling unit would be found adequate in the case of parenchyma, to give an estimate of this feature within a margin of 5% error of the mean. For sclerenchyma, however, it might have to be enlarged to bring down the sampling error to that percentage.

V. The Size of Cells in Ground Tissue

The size of parenchymatous cells forming the matrix, like the other two features, varies to a very great extent and is determined mainly by their position with respect to the cross-section of a stalk. In the peripheral zone, they are the smallest, arranged more or less regularly in rows, but as one proceeds from the periphery inwards, they progressively increase in size and become less regular in arrangement for some distance till they attain their maximum in respect of that variety. In the central region, unless otherwise affected by their proximity to a vascular bundle, they are more or less uniform in size. In view of the wide and rapid variation in size which is often of the order of 1 : 50 within a short distance of 2 mm., the determination of average size within a reasonable margin of error (5% of the mean) by direct methods, namely the measurement of cell-diameters under microscope or the measurement of area of camera lucida drawings with a planimeter, was not found to be a feasible proposition. To obtain an accurate idea of the cell-size by either method would involve a very large number of readings which might run into thousands. In the circumstances their average size in a particular zone was estimated by finding their number in unit area which obviously was inversely proportional to their size and was, therefore, a fairly reliable index of the latter. It is also evident that the number of cells which run into hundreds in a unit area ($0{\cdot}68^2$ sq. mm.) could not be found out by actually counting them under a microscope and had perforce to be arrived at by an indirect method. Therefore, a unit area square in shape having the diameter of a microscope-field as one of its sides was taken to facilitate easy calculation.

Because of rapid change in the size of cells in the peripheral region, two unit areas, an inner and an outer, adjacent to each other, were taken (Text-Fig. 8) and cells situated on the opposite sides of the square (tangential with respect to the cross-section of the stalk) were counted. Their average

was multiplied with the number of cells on the radial side to get the number of cells in unit area. For the outer unit, the epidermis formed outer of the two (tangential as already stated) sides of the square, while the inner unit was bounded towards the periphery by the inner side of the outer unit.



TEXT-FIG. 8

Since the cells were arranged more or less in rows, the area occupied by vascular bundles did not interfere with the estimation of their number in a unit area by this method. In the central region, the product of the two numbers obtained by counting cells on the adjacent sides of a square gave the requisite figure.

Data (Table XI) for three varieties were obtained for both the regions sectorwise, 5 observations per sector from the middle internode of three stalks.

From the table given above, it would appear that S.E. in a sample consisting of 60 observations, was 3·87–4·15% of the mean for the outer unit while for the inner unit it ranged between 4·49 and 5·87%. Further the estimated variance due to different zones when reduced to a percentage of the total variance, was also found to be more or less of the same order. The variation brought about by stalk-zone was almost negligible except in the Peshawar Paunda where it was respectively, 8·96% and 12·91% for the outer and inner units. Of the two other zones, namely the sector and cell, the former was much more important than the latter in the outer unit, while in the case of the inner unit, their contribution towards the total variance was more or less equal. In the Saharanpur Paunda, however, cell-zone acquired a dominant position. In the central region also, except in the Amritsar Paunda, the S.E. was very low and the total variance was due to the cell-zone alone. Therefore, in view of the low S.E. and the distribution of total variance among the zones in more or less the same proportion, it might be stated that the method suggested gave a fairly reliable estimate of the number of cells in unit area of matrix.

TABLE XI. *Showing estimated variance in the number of parenchymatous cells in unit area due to different zones in an initial sample and the size of required sample to give their number within a margin of 5% error*

Region	Varieties	Mean	S. E.	S. E. as % of the mean	$\sigma 1^2$	$\sigma 1^2$ as % of the total	$\sigma 2^2$	$\sigma 2^2$ as % of the total	$\sigma 3^2$	$\sigma 3^2$ as % of the total	No. of stalk with the corresponding no. of units						More than 6	
											1	2	3	4	5	6	Stalks	Units
Peripheral	Amritsar Paunda	1156·33	47·95	4·15	0·00	0·00	25594·55	71·94	9983·25	28·06		72	12	16	..	..	..	..
(*a*) Outer unit	Saharanpur Paunda	885·20	34·40	3·98	514·62	3·13	11201·13	68·14	4723·44	28·73		24	12	16	..	..	..	..
	Peshawar Paunda	902·27	34·91	3·87	1269·77	8·96	8707·36	61·47	4187·29	29·56		16	12	16	..	..	..	..
(*b*) Inner unit	Amritsar Paunda	147·13	7·73	5·25	21·45	2·08	535·08	51·82	476·02	46·10			204	32	40	..	..	..
	Saharanpur Paunda	138·55	6·22	4·49	0·00	0·00	299·33	26·71	821·14	73·29		80	36	32	..	..	..	..
	Peshawar Paunda	140·60	8·25	5·87	100·00	12·91	352·57	45·51	322·15	41·58				144	40	24	..	..
Central	Amritsar Paunda	142·00	16·07	11·32	713·45	53·95	..	..	608·97	46·05							15	225
																	16	112
																	17	85
	Saharanpur Paunda	121·80	3·94	3·23	0·00	0·00	..	..	233·07	100·00	7	8	9	..	..	..	..	..
	Peshawar Paunda	160·00	6·12	3·83	0·00	0·00	..	..	562·37	100·00	9	10	9	..	..	..	..	..

Note.—1. Unit area was a square, one of its sides in peripheral region being 0·68 mm. and in the central region 1.53 mm. in length.

2. The initial sample for both the outer and inner units in peripheral region was 60 unit areas each (3 stalks × 4 sectors × 5 units per sector) and for the central region 15 (3 stalks × 5 units per stalk).

3. $\sigma 1^2$ = Variance due to stalks; $\sigma 2^2$ = Variance due to sectors; $\sigma 3^2$ = Variance due to cells.

4. If the mean square due to a particular hierarchy happens to be less than that in the next one, the variance due to that hierarchy has been taken to be zero.

The initial sample of 3 stalks/60 observations was found to be more than ample for the outer unit of the peripheral region and gave a standard error in the neighbourhood of 4% of the mean. Therefore, the sample required for the estimation of this character within the allowable margin of error (5%) would be 3 stalks/12 observations. In the case of the inner unit, however, the initial sample was adequate for one variety only and a sample enlarged with respect to stalk but reduced as regards the number of observations per sector (5 stalks/40 observations) would cover all the three varieties. In the central region, 3 stalks/15 observations were more than sufficient for two varieties, the Amritsar Paunda being very wide off the mark.

Taking into consideration, the requirements of all the three units for this feature, and the sample-size, already arrived at for the two other characters, an initial sample of 3 stalks/36 observations, and 3 stalks/9 observations respectively for the peripheral and central regions was suggested to start with. As in the case of other characters, if data so collected on analysis were found to be inadequate for the purpose, the sample would have to be modified in the direction indicated by analysis.

Other pre-requisites to sampling for this character had already been dealt with while discussing them in connection with vascular bundle and thickness of cell-wall. In this case even the question of middle *versus* bottom internode did not arise because the cell-size attained its maximum only in a plant organ of normal dimensions.

VI. Summary

1. A sample for the comparative study of anatomical features of a sugarcane stalk, consists of three components, *viz.*, the stalk, the internode, and the anatomical character itself, each of which, if not selected according to a set plan, can vitiate results and thus render comparison invalid. The selection of a stalk, its number and its region (whether top, middle or bottom) for such studies is as important as the portion of an internode or the number of measurements taken for a particular character or the method of evaluating its magnitude.

2. The anatomical characters for which sampling procedure has been given and which amongst themselves cover the entire cross-section of a cane stalk are (*a*) the area of vascular bundles, (*b*) the thickness of cell-walls of parenchymatous and sclerenchymatous tissues and (*c*) the size of cells forming the parenchymatous ground tissue.

3. (*a*) Stalks of the same age should be compared. Mother shoots tagged at the time of emergence are the most suitable material for comparative study.

(*b*) Middle and basal internodes are equally representative of the stalk from which they are taken so far as the size of vascular bundles is concerned. But the stalk as a whole is better represented by the middle internode than by the basal one when the thickness of cell-walls is taken into consideration. Obviously a basal internode would give the maximum development of this feature while a middle internode would show the average for the whole stalk, the top internode occupying the other extreme. Parenchymatous cells forming the ground tissue acquire their maximum size only in an organ of normal dimensions which an internode attains in the middle of a stalk. Also greater length of a middle internode renders it easy to handle and section.

(*c*) In view of the stability of internal structure over considerable distance, the upper half of an internode should be sectioned.

4. (*a*) The standard error for a sample consisting of 3 stalks was found to be in the neighbourhood of 4% of the mean for vascular bundles and parenchymatous cells while for the thickness of cell-walls it usually did not exceed 10%.

(*b*) In this sample, variance due to stalk in the case of vascular bundles and parenchymatous cells was much below 5% of the total variance in the sample, whereas for the thickness of cell-walls it accounted for from 0·00% to 74·87% of the total, the range being much greater in the case of sclerenchyma.

(*c*) The contribution of sector to the total variance in vascular bundles was 0·00% in 7 out of 9 varieties. For the other two features, it was often the most important zone, more so for the parenchymatous cells and sometimes accounted for over 70% of the total variance.

5. (*a*) Since variance due to vascular bundles was invariably above 90% of the total, all of them in unit area would need to be measured in the peripheral zone. Their average size in the central region could be determined by measuring half the number found in unit area.

(*b*) The size of a vascular bundle as estimated by multiplying its radial and tangential axes measured under microscope, was found to bear a very high correlation, with the area of its figure. The correlation coefficients in peripheral and central regions were respectively $+0{\cdot}9699 \pm 0{\cdot}0107$ and $+0{\cdot}8889 \pm 0{\cdot}0488$.

(*c*) The total area of vascular bundles in unit area of ground tissue which was the summation of their individual areas, was found to be very convenient for comparison. Since it was not significantly different in the

four peripheral sectors of a transverse section of a cane, Sector No. 1 (having bud on the upper node) should be selected for making observations.

(*d*) Therefore, a sample for the estimation of the area under vascular bundles in peripheral region would consist of (i) all the vascular bundles found in (ii) twelve unit areas, taken four from each of Sector No. 1 of (iii) upper half of middle internode of (iv) three mother-shoots tagged at the time of emergence. In the central region also the number of unit areas would be the same, *i.e.*, four per stalk.

(*e*) The correlation coefficient between the average size of vascular bundles and their number per unit area of ground tissue ranged from $-0{\cdot}3607 \pm 0{\cdot}1422$ in Co 331 to $-0{\cdot}9463 \pm 0{\cdot}1078$ in Amristar Paunda. It was above $-0{\cdot}6$ in eight out of the fourteen varieties and significant at 1% level. This relationship, however, could not be put to much practical use because of the wide variation occurring in the magnitude of these attributes of vascular bundles as also in the total area under them.

6. (*a*) The thickness of cell-walls for parenchymatous and sclerenchymatous tissues would need to be measured in peripheral and central regions, the thickness for the former tissue being more or less the same upto 1·2 mm., from the epidermis.

(*b*) The sample-size for both the tissues would consist of 36 readings (3 readings per sector $\times$ 4 sector $\times$ 3 stalks) in peripheral region and 24 readings (8 readings per stalk $\times$ 3 stalks) in the central region.

7. (*a*) For the size of cells forming the parenchymatous matrix, their number in square unit area should be determined in the peripheral region (divided into two sub-regions) by multiplying the average number of cells situated on the two opposite sides (tangential with respect to the cross-section of stalk) of a square with that on the radial and in the central region by multiplying the number of cells on the adjacent sides.

(*b*) The size of a sample for this feature would be 36 observations (3 per sector $\times$ 4 sectors $\times$ 3 stalks), and 9 observations (3 per stalk $\times$ 3 stalks) respectively for peripheral (divided into two sub-regions) and central regions.

8. If on the analysis of data collected from the sample size suggested above, variance due to any of the three zones, namely stalk, sector and character, happens to acquire undue proportions, the sample would need to be modified in the direction indicated by analysis.

VII. Acknowledgments

The work was carried out at the Central Sugarcane Research Station, being jointly financed by the Bihar Government and the Indian Central Sugarcane Committee, to whom grateful thanks are due.

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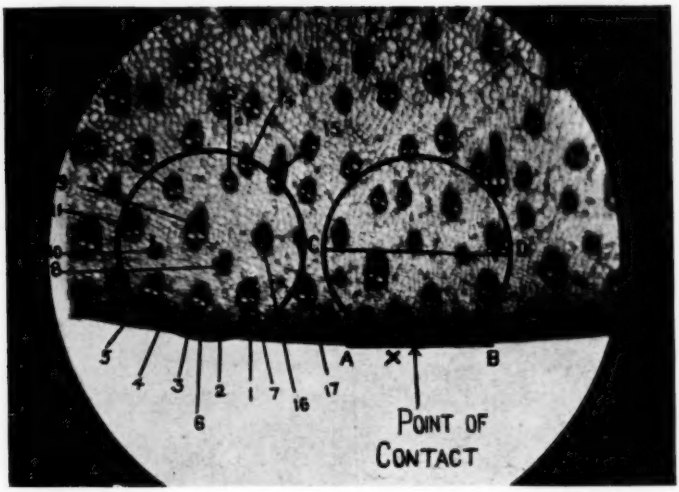
Explanation of Plate I

T.S. of Peshawar Paunda showing two unit areas of ground tissue in the peripheral region. In one of them, the vascular bundles bear the same number as those given in the Text-Fig. 2. Vascular bundle No. 11 is very near the diameter *CD* which is parallel to the tangent *AB* at the point of contact × between the epidermis and the edge of the microscope-field as shown in the other unit area. ×15.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APPENDIX

Relationship between the Size and Number of Vascular Bundles

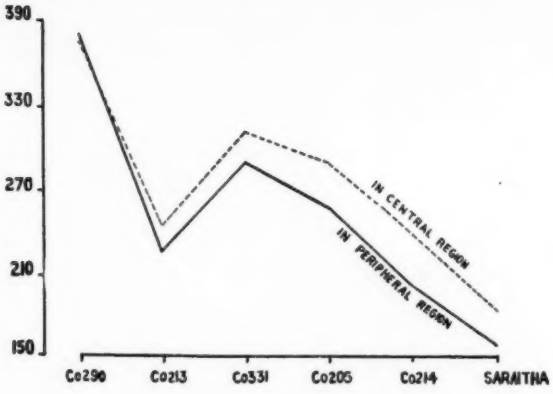
While working out the sampling technique, it often struck the authors that there existed a relationship between the number of vascular bundles in unit area of matrix and their average size; in other words if the number of the vascular bundles was small, they were comparatively large in size and *vice versa* (Table I, Text-Fig. 1).

Table I

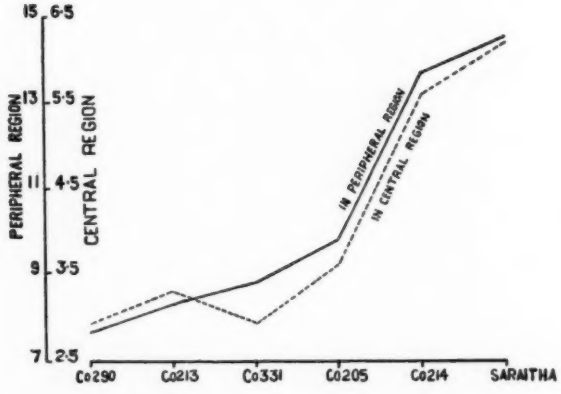
Showing the average size and number of vascular bundles per unit area

Variety	Peripheral Region		Central Region	
	No. of vascular bundles per unit area	Half the Mean Size	No. of vascular bundles per unit area	Half the Mean Size
Saccharum aitha ..	14·61 ± 0·74	80·85 ± 0·88	6·26 ± 0·26	92·36 ± 1·22
Co 205 ..	9·84 ± 0·31	158·25 ± 1·80	3·61 ± 0·22	175·68 ± 1·48
Co 213 ..	8·31 ± 0·17	114·11 ± 1·57	3·30 ± 0·12	121·57 ± 1·67
Co 214 ..	13·77 ± 0·17	103·17 ± 0·89	5·63 ± 0·13	120·22 ± ·132
Co 290 ..	7·67 ± 0·22	191·17 ± 2·54	2·88 ± 0·13	189·38 ± 2·32
Co 331 ..	8·80 ± 0·24	175·17 ± 1·97	2·91 ± 0·12	186·29 ± 2·33

This general impression was found to be roughly correct within wide limits as critical scrutiny of the data collected on 14 varieties showed that the relationship between the size and number of vascular bundles was not of the same order. The correlation coefficient ranged from $-0{\cdot}3607 \pm 0{\cdot}1422$ in Co. 331 to $-0{\cdot}9463 \pm 0{\cdot}1078$ in Amritsar Paunda, although in eight of the varieties examined it was above $-0{\cdot}6$ which was fairly high and



Average size of Vascular Bundles.



Average Number of Vascular Bundles per Unit Area.

TEXT-FIG. 1. (Appendix) Relation between average size and number of vascular bundles per unit area.

significant at 1% level (Table II). Only in two varieties, the correlation coefficient was below $-0{\cdot}5$.

TABLE II

Showing the number of vascular bundles per unit area, their average size and the total area under them in per unit area of ground tissue along with the correlation coefficient and regression equation of the first two features

Variety	No. of vascular bundles per unit area	Average size of vascular bundles	Total area of vascular bundles per unit area	Correlation coefficient	Regression equation. Where Y = Size of vascular bundles. X = Their number.
Co 205 ..	9·60	361·89	3407·97	−0·4690±0·1347	Y=669·86−32·08 X±9·2100
Co 213 ..	11·00	371·00	4143·42	−0·5861±0·1390	Y=600·09−19·95 X±4·2864
Co 214 ..	13·62	221·55	2996·67	−0·5429±0·1281	Y=353·94− 9·72 X±2·3001
Co 290 ..	7·76	414·84	3133·38	−0·6442±0·1116	Y=662·62−31·93 X±5·3331
Co 313 ..	11·78	325·72	4000·53	−0·6499±0·1303	Y=651·83−26·21 X±5·2583
Co 331 ..	8·73	374·43	3227·84	−0·3607±0·1422	Y=495·52−13·87 X±5·4699
Co 356 ..	13·97	399·75	5448·80	−0·6798±0·1258	Y=664·44−19·39 X±3·5889
Co 395 ..	12·17	346·57	4216·58	−0·5877±0·2559	Y=613·73−13·57 X±5·91
Co 453 ..	14·60	340·81	4970·08	−0·7840±0·1963	Y=621·13−19·04 X±4·77
Co 513 ..	13·60	309·67	4206·33	−0·6499±0·2403	Y=552·02−17·60 X±6·41
Saraitha ..	15·13	187·15	2767·50	−0·8650±0·0948	Y=326·04− 9·18 X±1·0059
Peshawar Paunda	13·25	205·04	2716·83	−0·5894±0·2554	Y=312·80− 8·10 X±3·4718
Saharanpur Paunda	13·67	196·58	2886·67	−0·7509±0·2088	Y=438·82−17·42 X±4·8456
Amritsar Paunda	16·27	193·86	3154·64	−0·9463±0·1078	Y=386·21−11·49 X±1·3075

Since the total area under vascular bundles was found to be different in different varieties, the authors were not able to put these conclusions to any practical use in reducing the number of observations. Of the three attributes of vascular bundles, namely the number, the average size and the total area under them in unit area of ground tissue, none was found to be constant or varying within narrow limits, and therefore determination of the magnitude of one of them did not give any accurate estimate of the other two.

STUDIES IN SAMPLING TECHNIQUE

Part II. Estimation of Pyrilla Incidence in Sugarcane

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1. Introduction

The problem of estimation of Pyrilla incidence on sugarcane (Plate II) in a given plot at a particular time has to be approached for solution through a sampling technique as in many other problems of estimation in biological sciences (Khanna, *et al.*, 1948). The main difficulty lies in the fact that there is no suitable direct method of measuring the incidence: it is not possible to measure the incidence by the amount of damage done by the pest to the crop, nor is it feasible to count the adult pest population present in the field. Recourse has, therefore, to be taken to an indirect method of recording the incidence (Khanna, 1944). Moreover, the notion of incidence and that of its intensity are so interconnected that it is not possible nor does it appear to be desirable to consider one with the exclusion of the other. *Prima facie*, it seems that the following characters taken together might give a full idea of the incidence and its intensity:—

(*a*) Percentage of leaf infestation (*a leaf being defined as infested or affected when there is an eggmass found laid on it*):—

Cane-wise figures in respect of:—

(i) Percentage of green leaf infestation on the basis of total green leaves.

(ii) Percentage of dry leaf and sheath infestation on the basis of total dry leaves.

(iii) Percentage of green leaf infestation on the basis of total dry leaves.

(*b*) No. of eggmasses:—

Cane-wise figures in respect of:—

(i) No. of eggmasses per affected cane.

(ii) No. of eggmasses per green leaf (healthy and affected), *i.e.* (Total No. of eggmasses present on the cane/Total No. of green leaves in the cane).

(iii) No. of eggmasses per affected green leaf.

(iv) No. of eggmasses per affected dry leaf.

All the above items have been considered simultaneously in the evolution of the sampling procedure, and it has been found that some of them are

not necessary for defining the incidence or its intensity in the data under consideration.

II. Material

Two plots, each measuring 60′ × 60′, infested with Pyrilla at Hanumangarh (Motihari) were completely enumerated in respect of all the details of the infested elements. Each row in a plot was divided into 3′-units and for each such unit full details of healthy and affected clumps, canes and leaves were available. From these records it is possible to evaluate all the indices enumerated above.

Table I shows the bulk of the data in the two plots. It will be noticed in the table that the number of clumps in the two plots is of the same order,

TABLE I. *Bulk of data and the details of infestation in the two plots*

Details	All canes		Affected canes	
	I	II	I	II
(1)	(2)	(3)	(4)	(5)
1 No. of rows ..	20	20	20	20
2 No. of 3′-units ..	399	400	399	400
3 No. of clumps ..	937	914	937	914
4 No. of canes ..	3819	2738	2664	1837
5 No. of leaves ..	36292 (D)	22397 (D)	26273 (D)	15637 (D)
	29919 (G)	21853 (G)	21319 (G)	15236 (G)
	66211	44250	47592	30873
6 No. of affected leaves ..	4519 (D)	3211 (D)	4519 (D)	3211 (D)
	493 (G)	46 (G)	493 (G)	46 (G)
	5012	3257	5012	3257
7 No. of eggmasses ..	11234 (D)	7531 (D)	11234 (D)	7531 (D)
	823 (G)	75 (G)	823 (G)	75 (G)
	12057	7606	12057	7606
8 %-age of canes affected	69·75	67·09	..	..
9 %-age of leaves affected	7·57	7·36	10·53	10·55
10 %-age of green leaves affected (basis total green leaves)	1·65	0·21	2·31	0·30
11 %-age of green leaves affected (basis green & dry leaves)	0·74	0·10	1·04	0·15
12 %-age of dry leaves affected	3·85 (L)	1·83 (L)	5·32 (L)	2·62 (L)
	8·14 (S)	12·42 (S)	11·24 (S)	17·78 (S)
	0·46 (L+S)	0·09 (L+S)	0·63 (L+S)	0·13 (L+S)
	12·45	14·34	17·20	20·53
13 No. of eggmasses per cane	3·16	2·78	4·53	4·14
14 No. of eggmasses per affected cane	4·53	4·14	4·53	4·14
15 No. of eggmasses per leaf	0·18	0·17	0·25	0·25
16 No. of eggmasses per green leaf	0·40	0·35	0·57	0·50
17 No. of eggmasses per affected green leaf	1·67	1·63	1·67	1·63
18 No. of eggmasses per affected dry leaf	2·49	2·35	2·49	2·35
19 No. of eggmasses per affected dry and green leaf	2·41	2·33	2·41	2·33

* Incidence of unit was not recorded according to programme.

whereas the number of canes in plot I is markedly more than in plot II. The number of leaves in plot I is also larger than in plot II, and the ratio of the numbers of leaves in the two plots is nearly the same as that of canes. This shows that the crop in plot I was more dense than that in plot II. The table* indicates that the percentages of canes affected in the two plots were 69·75 and 67·09 respectively. This means that roughly about two canes in three were affected in both the plots. The details of infestation furnished in Table I will further indicate that plot I is slightly more infested than plot II.

It will be apparent from the table that only a small fraction of green leaves was infested in both the plots. The percentages of infested green leaves have come out to be 1·65 and 0·21, calculated on the basis of total green leaves. The corresponding figures on the basis of total leaves are 0·74% and 0·10% respectively. If these percentages are worked out with reference to affected canes only, the percentage infestation still remains insignificantly small.

From the figures of percentage dry leaves infested, it will be clear that in an affected cane, mostly the sheath portions were infested; that is to say that eggmasses were found mostly on the sheath portions.

III. Indices Selected for Analysis

On account of the fact that the cane-wise values for the percentage green leaf infestation will be most frequently zero in a unit, the indices relating to green leaf infestation have been excluded and the following used to define incidence:—

(i) Percentage of dry leaves affected on the basis of dry leaves.

(ii) No. of eggmasses per affected cane.

(iii) No. of eggmasses per green leaf (Total No. of eggmasses in the cane/Total No. of green leaves in the cane).

(iv) No. of eggmasses per affected dry leaf.

If the calculation of these items is referred to individual canes in the unit, the cane-wise figures in respect of items (i) and (iii) will also frequently be zero. As a matter of fact, about 3 values out of 10 in plot I and 2 values out of 7 in plot II on the average will be zeroes. The above items have, therefore, been calculated in the first instance with reference to affected canes only (as it was proposed to define incidence on the basis of canes), and subsequently with reference to " all canes " as well. In the latter case, a pooled incidence has been calculated for each unit.

* All the values furnished in the table are pooled for the whole plot.

IV. The Type of Sampling Adopted

(i) *The first category.*—Out of the units available in the two plots, random selection has been made of 2½%, 5% and 10% units in the first instance and from each unit 4 affected canes have been selected at random. Since in plot II, the number of affected canes was not larger than 5 on the average, a few of the randomised units had to be rejected, and fresh random units taken to get 4 affected canes in each unit.

(ii) *The second category.*—In case of the incidence figures referred to "all canes", there was no necessity of randomising canes in a unit, as the calculation of incidence has been done on the unit basis. Under this category, fresh selection was made of 2½%, 5% and 10% units and each size was repeated thrice in each plot.

V. The Estimates of the Variances

The mode of sampling referred to in 4 (i) obviously leads to "twofold" nested sampling where the variance of the mean yield would be given by

$$V(x..) = \frac{\Sigma n_i^2}{n^2}\sigma_1^2 + \frac{1}{n}\sigma_2^2,$$

where n_i is the number of canes in the ith unit and $\Sigma n_i = n$ (total No. of canes) and σ_1^2 and σ_2^2 are respectively the estimates of the true variances between units and between canes. If V_1 be the mean square between units and V_2 that between canes, V_2 will be the unbiased estimate of σ_2^2 and V_1 the unbiased estimate of

$$\frac{n - \frac{\Sigma n_i^2}{n}}{t-1}\sigma_1^2 + \sigma_2^2,$$

where t is the number of units taken in the sample. If, however, an equal number of canes is selected in each unit, the variance of the mean incidence would reduce to

$$V(x..) = \frac{\sigma_1^2}{t} + \frac{\sigma_2^2}{tf}$$

where t is the number of units and f the number of canes per unit.

VI. Nature of Variations Obtained in the First Category and the Discussion of Results

Table II shows the estimates of the variances due to units and canes along with the percentage standard errors. It will be noticed in the columns showing the percentage variations that canes have contributed more than 80% of the total variations on the average. The table will indicate that of the

TABLE II. *Estimates of the variances between units and between canes (taki 4 can*

Characters	2½%						
	σ_1^2	%	σ_2^2	%	Mean ± S. E.	S. E expres as % the me	σ_1^2
Col. (1)	col. (2)	col. (3)	col. (4)	col. (5)	col. (6)	col. (7	col. (8)
1. %-age of dry leaves affected on the basis of dry leaves	33·5711	14·22	202·4522	85·78	21·50±2·90	13·4	15·6329
	46·0907	14·23	277·7094	87·77	23·57±3·40	14·4	15·0667
2. No. of eggmasses per affected cane	0·44	2·84	15·03	97·16	4·95±0·65	13·1	0·76
	7·98	44·46	9·97	55·54	4·45±1·25	28·0	2·97
3. No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the cane)	0	0	0·2404	100·00	0·62±0·08	12·9	0·0346
	0·1312	38·98	0·2054	61·02	0·57±0·14	24·8	0·0425
4. No. of eggmasses per affected dry leaf	2·2217	28·75	5·5067	71·25	2·87±0·59	20·5	0·6024
	0·3404	8·18	3·8198	91·82	2·48±0·36	14·3	0

Upper figures stand for Plot No. I and those below for Plot No. II.

4 characters, the first character, namely the percentage of dry leaves affected has shown consistently the lowest variation and that plot II has shown invariably a larger variation in incidence in each of the characters.

The standard errors expressed as percentage of the mean have been very high for 2½% of the units. Even for 5% of the units, the standard errors have been more than 7·44% in all the characters. The standard errors with 10% of the units have been the lowest, but, none-the-less they have exceeded 5% of the mean in all the characters except for one character (the 1st character) in plot (I).

These standard errors were calculated on the basis of 4 affected canes per unit. Mention was made elsewhere in the note that on the average 7 canes in plot I and 5 canes in plot II were affected per unit. If, however, the standard errors are calculated making use of these variances and varying the number of canes per unit as 7 in plot I and 5 in plot II, the standard errors show some reduction but the reduction hardly exceeds 1%. These percentage standard errors have been shown in Table III and it will be noticed that only in respect of the first character, the standard errors have been less than 5% of the mean, and that too, with 10% units only. This leads us to the question as to how many units have to be selected to get the errors within 5% of the mean and also to the query as to what would have been the error, had it been calculated on the basis of the complete data available in the two plots.

es (taki 4 canes per unit) S. E. expressed as the percentage of the means (affected canes only)

		5%						10%					
E.	S. E. expressed as % of the mean col. (7)	σ_1^2 col. (8)	% col. (9)	σ_2^2 col. (10)	% col. (11)	Mean ± S. E. col. (12)	S. E. expressed as % of the mean col. (13)	σ_1^2 col. (14)	% col. (15)	σ_2^2 col. (16)	% col. (17)	Mean ± S. E. col. (18)	S. E. expressed as % of the mean col. (19)
	13·45	15·6329	11·45	120·9525	88·55	20·29±1·51	7·44	5·7178	6·75	79·0105	93·25	20·17±0·79	3·92
	14·43	35·0667	32·95	71·3514	67·05	21·74±1·63	7·50	15·9486	11·91	117·9869	88·09	21·18±1·07	5·05
	13·12	0·76	7·01	10·08	92·99	4·48±0·40	8·93	1·00	6·20	15·12	93·80	5·23±0·35	6·69
	28·09	2·97	17·92	13·60	82·08	4·11±0·56	13·63	2·47	16·34	12·65	83·66	4·04±0·37	9·16
	12·98	0·0346	2·71	0·1325	97·29	0·56±0·06	10·71	0·0327	1·74	0·2718	98·26	3·67±0·05	7·46
	24·58	0·0425	16·79	0·2106	83·21	0·51±0·07	13·73	0·0354	16·97	0·1732	83·03	0·49±0·04	8·16
	20·58	0·6024	16·90	2·9621	83·10	2·62±0·26	9·92	0·8966	25·57	2·6102	74·43	2·68±0·19	7·09
	14·53	0	0	8·6200	100·00	2·46±0·33	13·41	0·0936	2·89	3·2395	97·11	2·33±0·15	6·44

* Denotes significance from the population value at 1% level.

TABLE III. *S. E. expressed as percentages of the mean when 7 canes and 5 canes are taken in a unit respectively in the two plots (affected canes only)*

Characters		2½%		5%		10%	
		Mean ± S.E.	S.E. expressed as the %-age of the mean	Mean ± S.E.	S.E. expressed as the %-age of the mean	Mean ± S.E.	S.E. expressed as the %-age of the mean
(1)		(2)	(3)	(4)	(5)	(6)	(7)
1 % of dry leaves affected on the basis of dry leaves	1	21·50±2·50	11·63	20·29±1·28	6·31	20·17±0·65	3·22
	2	23·57±3·19	13·53	21·74±1·57	7·22	21·18±0·99	4·67
2 No. of eggmasses per affected cane	1	4·95±0·51	10·30	4·48±0·33	7·37	5·23±0·28	5·35
	2	4·45±0·99	22·44	4·11±0·53	12·90	4·04±0·35	8·66
3 No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the canes)	1	0·62±0·59	9·52	0·56±0·52	9·29	0·67±0·04	5·97
	2	0·57±0·13	22·81	0·51±0·06	11·76	0·49±0·04	8·16
4 No. of eggmasses per affected dry leaf	1	2·87±0·55	9·16	2·62±0·23	8·78	2·68±0·18	6·72
	2	2·48±0·33	13·31	2·46±0·29	11·79	2·33±0·14	6·01

The entire data in the two plots have been analysed to find the variances between units and between canes. Table IV will show the results of analysis.

TABLE IV. *Summary of population values (affected canes only)*

Characters	σ_1^2		σ_2^2		Mean ± S.E.	S.E. expressed as the % of the mean
1 %-age of dry leaves affected on the basis of dry leaves	20·0900	16·83%	99·2900	83·17%	17·78±0·3212	1·81
	40·6700	26·84%	110·8600	73·16%	21·58±0·4455	2·06
2 Population of eggmasses per affected cane	2·2400	27·65%	5·8600	72·35%	4·94±0·0969	1·96
	3·0677	21·34%	11·3100	78·66%	4·03±0·1288	3·20
3 No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the canes)	0·0251	7·49%	0·3100	92·51%	0·60±0·0141	2·35
	0·0458	16·04%	0·2398	83·96%	0·53±0·0169	3·11
4 Population of eggmasses per affected dry leaf	0	0·00%	5·9102	100·00%	2·82±0·0485	1·72
	0·6019	12·87%	2·5200	88·13%	2·26±0·0585	2·59

* Upper figures for Plot No. I and those below for Plot No. II.

The differences in the mean values obtained from the samples and from the " whole " plots have been tested for statistical significance and it has been found that the sample mean only in one case has been significantly different from the whole plot value. This sample estimate has been marked with an asterisk (*) in Table II.

It will be noticed in Table IV that canes have contributed on the average about 84% of the total variation and that this finding is consistent with what has been obtained before in the samples. The standard errors expressed as percentages of the mean vary from 1·72 to 3·11 in the different characters. The inherent variation is therefore already large and consequently if a precision to the extent of 5% of the mean is desired, the sample size would perhaps be too large for practical purposes.

In Table V has been shewn the number of units to be included in the sample so that the error may not exceed 5% and 7% of the mean and these numbers have been worked out taking 4 canes per unit in both the plots in the first instance and also taking 7 canes per unit in plot I and 5 canes in plot II. The latter two numbers have been taken to see as to what should be the number of units if the units were completely enumerated with regard to all the affected canes. The number of units to be selected should follow more or less the same trend irrespective of the sample size ($2\frac{1}{2}$%, 5%, or 10% units), from which the variances have been taken to work out these numbers. That there has been some variation in the trend is mainly due to the fact that the estimates of σ_1^2 came out to be zeroes in two samples and that the estimates of the variances obtained from larger sample sizes had more

TABLE V. *No. of units to be selected so that the S. E. may be 5% and 7% of the mean in the different cases*

Characters	4 canes in a unit						7 canes in plot No. I and 5 canes in plot No. II in a unit						Remarks
	2½%		5%		10%		2½%		5%		10%		
1	I 2	II 3	I 4	II 5	I 6	II 7	I 8	II 9	I 10	II 11	I 12	II 13	14
1 %-age of dry leaves affected on the basis of dry leaves	72 37	84 43	45 23	46 14	25 13	41 21	54 28	74 38	33 17	43 13	17 9	36 19	
2 No. of eggmasses per affected cane	70 35	219 109	69 34	152 76	71 35	141 72	44 22	208 104	46 23	136 68	47 23	125 65	
3 No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the cane)	63 38	231 113	67 38	133 57	84 40	126 65	32 19	218 107	56 32	118 50	59 28	113 59	
4 No. of eggmasses per affected dry leaf	180 90	93 45	80 42	155 74	92 43	65 34	151 76	79 38	61 32	123 60	75 36	46 24	

* Upper figures leading to the precision of 5% of the mean and those below to that of 7% of the mean.

precision on account of more degrees of freedom being available for the estimation of the variances.

It will be reasonable, therefore, to make use of the variances obtained from the size of 10% units in deciding the question of the number of units to be selected in a sample. Fixing the level of precision at 7% of the mean and enumerating all the affected canes in a unit, the numbers of units to be selected for the four characters come out to be 9, 23, 28, 36; and 19, 65, 59, 24 respectively for the two plots. On account of the fact that the variation in plot II was larger than that in plot I, the requirement of the number of units also has been greater in plot II than in plot I. The second and third characters require in plot II 65 and 59 units or 16·25% and 14·75% respectively. The first and the fourth items require respectively 19 and 24 units which work out as 4·75% and 6·00% of the total units. If precision is desired at the same level of 7% uniformly in all the characters, the number of units should be 65 or 16·25% of the total units available.

VII. The Nature of Variation in the Second Category and the Discussion of Results

The estimates and the standard errors expressed as percentage of the estimates for each sample in the second category have been furnished in

Table VI. Three samples were drawn under each size in respect of each of the four characters and the variations between different samples in the same character as compared against the variations within samples have been shewn in Table VII. It will be noticed in Table VII that the variation between samples is non-existent as compared to the variation within samples. This shows that the estimated incidences obtained from the different samples are consistent and therefore dependable. Test of significance reveals that only in 3 samples the mean values have shown significant differences from the population values. Though the calculation of standard errors shown in

TABLE VI. *Estimates of the standard errors expressed a*

Characters (1)		2½%					
		Mean ± S.E. I (2)	% (3)	Mean ± S.E. II (4)	% (5)	Mean ± S.E. I (6)	% (7)
1. %-age of dry leaves affected on the basis of dry leaves	1	13·14±2·16	16·44	13·52±2·54	18·79	12·91±1·78	13·79
	2	14·14±2·16	15·28	17·43±2·54	14·57	10·78±1·78	16·51
	3	12·07±2·16	17·90	14·08±2·54	18·04	11·41±1·78	15·60
2. No. of eggmasses per affected cane	1	4·06±0·67	16·50	4·47±0·82	18·34	4·51±0·66	14·63
	2	4·68±0·67	14·32	4·33±0·82	18·94	4·15±0·66	15·90
	3	4·31±0·67	15·55	3·88±0·82	21·13	4·50±0·66	14·67
3. No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the canes)	1	0·32±0·08	25·00	0·77±0·23	29·87	0·38±0·05	13·16
	2	0·42±0·08	19·05	0·41±0·23	56·10	0·40±0·05	12·50
	3	0·38±0·08	21·05	0·31±0·23	74·19	0·41±0·05	12·20
4. No. of eggmasses per affected dry leaf	1	2·49±0·38	15·26	2·52±0·32	12·70	2·45±0·90	36·73
	2	2·52±0·38	15·08	2·30±0·32	13·91	2·79±0·90	32·26
	3	2·63±0·38	14·45	2·12±0·32	15·09	2·55±0·90	35·29

TABLE VII. *Estimates of the variances between samples and between*

Characters (1)	2½%					
	σ_1^2 (2)	% (3)	σ_2^2 (4)	% (5)	Mean ± S.E. (6)	% (7)
1. %-age of dry leaves affected on the basis of dry leaves	0	0	46·7028	100·00	13·11±1·10	8·39
	0	0	64·5909	100·00	15·01±1·47	9·79
2. No. of eggmasses per affected cane	0	0	4·4692	100·00	4·35±0·39	8·97
	0	0	6·6813	100·00	4·23±0·47	11·11
3. No. of eggmasses per green leaf (Total eggmasses in a cane/Total green leaves in the canes)	0	0	0·0591	100·00	0·37±0·04	10·81
	0·0056	1·06	0·5243	98·94	0·50±0·14	28·00
4. No. of eggmasses per affected dry leaf	0	0	1·4505	100·00	2·54±0·22	8·66
	0	0	1·0479	100·00	2·31±0·19	8·23

Table VI has been done with the unit-wise incidences, these standard errors are not different in nature from those indicated in Tables II and III. If the mean value obtained from the cane-wise incidences of a unit would have been taken to represent the incidence of the unit, the standard error calculated on the basis of these incidences as done under Table VI would have been exactly equal to that found under the twofold nested sampling. The standard errors in Table III were calculated on the basis of the number of all affected canes and as such these standard errors would be comparable with those in Table VI where the incidences were calculated having regard

percentages of the means in the different samples (all canes)

5%		10%				Population value			
Mean ± S.E. II (8)	% (9)	Mean ± S.E. I (10)	% (11)	Mean ± S.E. II (12)	% (13)	Mean ± S.E. I (14)	% (15)	Mean ± S.E. II (16)	% (17)
15·63±1·17	7·49	12·67±0·91	7·18	15·43±1·06	6·87	12·81±0·30	2·34	15·18±0·35	2·31
13·47±1·17	8·69	13·56±0·91	6·71	14·46±1·06	7·33				
13·00±1·17	9·00	13·47±0·91	6·76	14·85±1·06	7·14				
4·02±0·26	6·47	4·70±0·29	6·17	†5·06±0·43	8·50	4·44±0·10	2·25	3·84±0·11	2·86
†3·03±0·26	8·58	4·55±0·29	6·37	4·48±0·43	9·60				
3·58±0·26	7·26	4·39±0·29	6·61	4·41±0·43	9·75				
0·35±0·04	11·43	0·42±0·03	7·14	0·37±0·04	10·81	0·43±0·01	2·33	0·37±0·01	2·70
0·27±0·04	14·81	0·41±0·03	7·32	0·38±0·04	10·52				
0·29±0·04	13·79	0·41±0·03	7·32	0·38±0·04	10·53				
2·12±0·13	6·13	2·62±0·16	6·11	2·24±0·20	8·93	2·54±0·06	2·36	2·19±0·05	2·28
*1·89±0·13	6·88	2·56±0·16	6·25	2·50±0·20	8·00				
2·40±0·13	5·42	2·46±0·16	6·50	2·42±0·20	8·26				

* Denotes significance at 5% level. † Denotes significance at 1% level.

units: S. E. expressed as the percentages of the means (all canes)

5%						10%					
σ_1^2 (8)	% (9)	σ_2^2 (10)	% (11)	Mean ± S.E. (12)	% (13)	σ_1^2 (14)	% (15)	σ_2^2 (16)	% (17)	Mean ± S.E. (18)	% (19)
0	0	31·7207	100·00	11·70±0·73	6·24	0	0	33·0069	100·00	13·23±0·52	3·93
0·5851	2·08	27·5245	97·92	14·03±0·81	5·77	0	0	44·5906	100·00	14·91±0·61	4·09
0	0	4·4109	100·00	4·39±0·27	6·15	0	0	3·4564	100·00	4·55±0·17	3·74
0·1769	11·43	1·3711	88·57	3·54±0·29	8·19	0	0	7·3705	100·00	4·32±0·25	5·79
0	0	0·0553	100·00	0·40±0·03	7·50	0	0	0·0484	100·00	0·41±0·02	4·88
0	0	0·0309	100·00	0·30±0·03	10·00	0	0	0·0815	100·00	0·38±0·03	7·89
0	0	1·6307	100·00	2·59±0·16	6·18	0	0	1·0657	100·00	2·54±0·09	3·54
0·0461	11·31	0·3614	88·69	2·14±0·15	7·01	0	0	1·5697	100·00	2·38±0·11	4·62

σ_1^2 = Between samples. σ_2^2 = Within samples.

to all the affected elements in a unit. Comparison of the standard errors reveals that if the enumeration of all the affected elements in a unit be thought desirable, it would perhaps be slightly more advantageous to calculate incidence on the basis of canes for the sake of precision.

VIII. Summary of Conclusions

1. Two plots each, measuring 60′ × 60′ infested with Pyrilla were completely enumerated to find the minimum sample size for the estimation of incidence at a desired level of precision. Of the two plots, plot I had a denser crop and a slightly more of infestation than plot II. But the variation of incidence in plot II has been of a slightly higher order than in plot I.

2. Four characters have been simultaneously used to define incidence and the sample sizes have been worked out in terms of the percentages of the total 3′-units available in the plots. The four characters and the sample size in respect of each are enumerated below. The sample sizes are such as would furnish the estimates within an error of 7%.

Characters	Percentage of 3′-units
(i) Percentage of dry leaves affected on the basis of dry leaves	4·75
(ii) No. of eggmasses per affected cane	16·25
(iii) No. of eggmasses per green leaf (total No. of eggmasses on the cane/total No. of green leaves in the cane)..	14·75
(iv) No. of eggmasses per affected dry leaf	6·00

For the percentage units stated above, the estimation of incidence has to be based on the cane-wise figures for all the affected canes in the units.

IX. Acknowledgment

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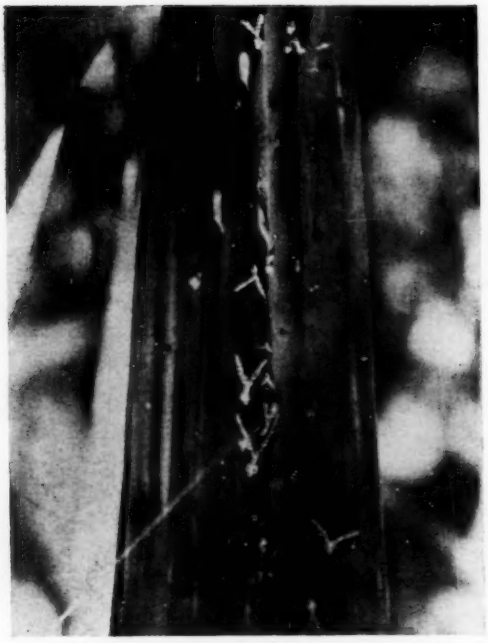
A field photograph showing incidence of Pyrilla in Sugarcane : *a*, moth ; *b*, nymph ; *c*, egg masses.



A close up view of a portion of sugarcane leaf showing moth and nymphal population

SOME STUDIES ON THE INFLUENCE OF SPACING, SEED PIECE AND MANURING ON THE YIELD OF POTATO CROP

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Introduction

Seed rate varies greatly in potato growing areas. It ranges from 3 maunds in Bihar where the seed tubers commonly used for planting vary from 1/6 inch to 1 inch and 24 maunds in Madras where the size of tuber sown is 1 inch to 1¾ inches. Whole tubers as well as cut pieces are used by growers. An investigation was, therefore, undertaken to inquire into the relative advantages of using large seed rates in preference to small seed rates.

The experimental evidence from the work of Adams (1889), Aicher (1917), Zavitz (1916) and Bates (1935), indicates that there is a direct correlation betweeen the size of seed and yield of tubers, higher yields being obtained from large seed pieces. These studies were, however, conducted at one level of fertility and the nature of response under varying levels of manuring is not precisely known. The present work is intended to add to our knowledge the effects of seed piece, spacing and manuring with a view to find the size of seed piece and the amount of seed rate conducive to high yields and better net returns.

Methods

The experiment was laid out on the College Experimental Farm in 1945-46 and 1947–48. The system of layout consisted of Randomised blocks with 3 replications of 27 treatments each. The size of gross plot was 18′ × 24′ and ultimate plot 14′ × 20′, containing respectively 12 and 10 rows spaced 2 feet apart.

Treatments

There were three factors with three treatments under each.

1. *Size of seed piece.*—Seed potatoes *var.* Darjeeling Red, grade 1½ inches were obtained through the Directorate of Agriculture, West Bengal,

and subsequently sorted to select potatoes of 20 gm. weight. These were sown whole, half and quarters weighing 20, 10 and 5 gm. respectively.

(i) 5 gm. set—W_1; (ii) 10 gm. set—W_2; (iii) 20 gm. set—W_3.

2. *Spacing.*—The distance between rows was kept uniformly at 2 feet and variation was made only in the spacing between hills.

(i) 4″ spacing—S_1; (ii) 8″ spacing—S_2; (iii) 12″ spacing—S_3.

The rate of planting as calculated with the size of seed piece and distance in the row is given in Table I.

TABLE I

Quantity of potatoes in maunds required to plant one acre at different spacings with seed piece of various sizes

Spacing between hills	Size of seed piece		
	5 gm.	10 gm.	20 gm.
4 inches ..	8·79	17·58	35·16
8 ..	4·39	8·79	17·58
12 ..	2·93	5·86	11·72

3. *Manuring.*—Manuring consisted of basal dressing with cow-dung followed by top application of ammonium sulphate.

(i) No manure—M_1; (ii) 250 maunds of cow-dung + 100 lb. of ammonium sulphate/acre—M_2; (iii) 500 maunds cow-dung + 200 lb. of ammonium sulphate/acre—M_3.

Treatment combinations

There were 27 treatments obtained by all combinations of seed piece, spacing and manuring.

Size of seed piece × Spacing between hills × Levels of manuring.

W_1	S_1	M_1
W_2	S_2	M_2
W_3	S_3	M_3

Collection of data

The crop was harvested at maturity. The field and plot borders were lifted first and ignored for purposes of collection of the data. Experimental

observations were confined to ultimate plots only. The following characters were studied:

1. Yield of tubers per plot.

2. Average size of tubers—Plot yield was divided by the total number of tubers obtained from the respective plots.

Experimental Findings

Yield studies

The yield of different treatment combinations is presented in Table II. Wide variation is noticed under the various treatment combinations; the lowest yield of 175 ounces is obtained from $W_1S_3M_1$ and the highest of 1466 ounces is recorded under the treatment combination of $W_3S_1M_3$.

Table II

Yield of potatoes per plot in ounces under the treatment combinations of size of seed piece, spacing between hills and levels of manuring (1945-46)

	S_1			S_2			S_3		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
M_1 ..	273	305	338	200	365	527	175	241	332
M_2 ..	576	756	794	580	925	961	406	628	647
M_3 ..	1357	1441	1466	961	982	1053	636	762	762

S. E. of a mean .. .. = 38·74 C. D. at 5% = 109·92
S. E. of a difference of means .. = 54·77 C. D. at 1% = 146·50

Main effects.—Maximum response is made to the levels of manuring (Table III). M_3 gives a yield of 1047·0 ounces while M_1 yields only 306·7 ounces per plot. A comparison of yield under variations of size of seed piece shows a regular increase when the seed piece is changed from small to large. Unlike the effects of manuring and seed piece, increase of spacing causes a depression in yield values. The differences obtained under all the three factors are highly significant.

Interactions.—The data pertaining to first order interactions are presented in Table III. Variations in size of seed piece affects the yield but the degree of effectiveness varies under the different levels of manuring. The increase in yield of large over small seed piece is 183·1 ounces under M_1, but only 108·9 ounces under M_3 level. It is of interest to note that the increase due to seed piece under M_3 though significant is relatively small.

TABLE III

Yield of potatoes per plot in ounces under the main effects and first order interactions of size of seed piece, spacing between hills and levels of manuring (1945-46)

	W_1	W_2	W_3	Mean	Increase
M_1 ..	216·4	304·3	399·5	306·7	
M_2 ..	521·0	770·2	800·7	697·3	+390·6
M_3 ..	985·0	1062·2	1093·9	1047·0	+349·7
Mean ..	574·1	712·2	764·7		
Increase ..		+138·1	+52·5		
	S_1	S_2	S_3	Mean	Increase
M_1 ..	306·0	364·4	249·8	306·7	
M_2 ..	709·0	822·3	560·6	697·3	+390·6
M_3 ..	1421·6	998·9	720·5	1047·0	+349·7
Mean ..	812·2	728·5	510·3		
Increase ..		−83·7	−218·2		
	S_1	S_2	S_3	Mean	Increase
W_1 ..	735·6	580·6	406·1	574·1	
W_2 ..	834·6	757·8	544·3	712·2	+138·1
W_3 ..	866·3	847·2	580·6	764·7	+ 52·5
Mean ..	812·2	728·5	510·3		
Increase ..		−83·7	−218·2		

S. E. of increase – Main effects = 18·26
S. E. of increase – 1st order interactions = 31·61
C. D. at 5% – Main effects = 36·64
C. D. at 5% – 1st order interactions = 63·44

Interaction between spacing and manuring indicates that at low and medium manuring, the spacing of 8 inches is more suitable; both below and beyond 8 inches spacing, the yield falls down. The differences are generally significant. The response under M_3 is, however, different and the highest yield is recorded under 4 inches spacing; the plot yields under the three spacings being 1421·6, 998·9 and 720·5 ounces respectively. With high manuring, close planting seems more desirable.

The manuring variable holds its significant influence under all the levels of seed piece and spacing, its response remaining unaffected under any change of either seed piece or spacing.

Interaction between seed piece and spacing shows that yield increases with increase of the size of seed piece and it decreases with increase of spacing between hills.

Perusal of Table II further indicates that yield is rather small under low manuring and considering the cost spent on seed, potato growing under

poor fertility seems undesirable. When manuring is moderate the use of 10 gm. seed piece sown 8 inches apart is found more useful. The yield and the gross income is, of course, higher under S_2W_3 but examining the seed rate (Table I) and seed costs it is seen that the small addition in yield is inadequate to compensate for the extra cost spent on seed. Under heavy manuring the highest yield is recorded from S_1W_3 but as regards actual net returns 5 gm. seed piece sown 4 inches apart gives more profit. It may be stated that the price of seed potatoes ranged from Rs. 30 to Rs. 50 and that of table potatoes at harvest time from Rs. 5 to Rs. 7 per maund in the quinquennium ending 1948.

The general trend of results for the year 1947-48 is similar to that described above.

Average tuber weight

The relative tuber weight under the various treatments is presented in Table IV. The smallest size is produced by the 20 gm. seed piece spaced 4 inches apart and grown under the lowest level of fertility; the largest on the other hand, is obtained from the seed piece of 5 gm. planted 12 inches apart and raised under the highest level of manuring.

TABLE IV

Average size of tuber in gm. under the treatment combinations of size of seed piece, spacing between hills and levels of manuring (1945-46)

	S_1			S_2			S_3		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
M_1 ..	6·47	6·18	5·34	9·03	9·06	8·80	11·33	9·42	8·92
M_2 ..	10·67	9·09	8·29	19·22	18·65	16·67	20·29	18·28	17·44
M_3 ..	18·92	15·43	13·38	22·93	18·81	16·71	23·38	20·86	17·78

S. E. of a mean .. .. = 0·49 C. D. at 5% = 1·38
S. E. of a difference of means .. = 0·69 C. D. at 1% = 1·84

Main effects.—Maximum response is made to the manuring factor (Table V). The size of tuber is small under low manuring and it improves significantly with each increase in the rate of manuring. Thus the size of tuber which is only 8·28 gm. under low manuring, increases to 15·40 gm. under medium manuring and develops to give 18·69 gm. when manuring level is raised to M_3.

TABLE V

Average size of tuber in gm. under the main effects and first order interactions of size of seed piece, spacing between hills and levels of manuring (1945-46)

	W_1	W_2	W_3	Mean	Increase
M_1 ..	8·94	8·22	7·69	8·28	
M_2 ..	16·73	15·34	14·13	15·40	+7·12
M_3 ..	21·77	18·37	15·95	18·69	+3·29
Mean ..	15·81	13·97	12·59		
Increase ..		−1·84	−1·38		
	S_1	S_2	S_3	Mean	Increase
M_1 ..	6·00	8·96	9·89	8·28	
M_2 ..	9·35	18·18	18·67	15·40	+7·12
M_3 ..	15·93	19·48	20·67	18·69	+3·29
Mean ..	10·42	15·54	16·41		
Increase ..		+5·12	+0·87		
	S_1	S_2	S_3	Mean	Increase
W_1 ..	12·04	17·06	18·33	15·81	
W_2 ..	10·23	15·51	16·19	13·97	−1·84
W_3 ..	9·00	14·06	14·71	12·59	−1·38
Mean ..	10·42	15·54	16·41		
Increase ..		+5·12	+0·87		

S. E. of increase – Main effects = 0·22
S. E. of increase – 1st order interactions = 0·39
C. D. at 5% – Main effects = 0·44
C. D. at 5% – 1st order interactions = 0·78

The effect of size of seed piece is much different than that of manuring. A large size is obtained from small seed piece and conversely a small size is produced by the large seed piece. The values under the small, medium and large seed piece are 15·81, 13·97 and 12·59 gm. respectively. The differences are highly significant.

Response to increase in spacing is similar to that of manuring. Closer spacing yields small potatoes and a significant improvement is effected by increasing the distance between hills.

Interactions.—The effect of seed piece remains more or less same under all the levels of manuring; small seed piece gives better size of potatoes and it continues to do so under low, medium, and high manuring (Table V). The increase in size by 5 gm. over 20 gm. seed piece is 1·25 gm. under M_1, 2·60 gm. under M_2 and 5·82 gm. under M_3 levels of manuring.

The response to spacing also remains unaltered whether the manuring level is low or high; wide spacing yields better grade of potatoes and a reduced size is obtained when planting is made 4 inches apart.

The level of manuring maintains its effects under all sizes of seed piece and spacings between hills. Each increase in the rate of manuring results in a corresponding significant improvement in the grade of potatoes.

The study of interactions between size of seed piece and spacing indicates that both maintain their individual effect under all the levels of the other factor. Large spacing yields relatively better size of potatoes whether sowing is done with small, medium or large seed piece. Similarly large seed piece yields potatoes of smaller size irrespective whether the planting distance is 4 inches, 8 inches or 12 inches.

Data for the year 1947-48 are in close accord with the results given above.

Discussion

Yield increases by raising the level of fertility, increasing the size of seed piece and decreasing the spacing between hills.

Response to fertility for higher yield is well established and is shown clearly in Tables II and III. As regards size of seed, the amount of food material in the mother tuber is an influencing factor. Substances stored in the mother tuber are utilised by the sprout. Thus bigger the seed piece greater the initial food supply. Appleman (1918), Bates (1935), Hume *et al.* (1914), Hutcheson and Wolfe (1917) and Zavitz (1916) have shown that the vigour of the plant, the subsequent rate of growth and the final yield are directly related to the size of seed piece. Spacing affects the yield by its influence in controlling the density of population of hills. The number of hills per plot is 420 with 4 inches, 210 with 8 inches and only 140 when planting is done 12 inches apart. The relative yield per hill is more under wide spacing (Table VI) but the added increase in yield is too small to compensate for the decrease in the number of hills. The yield, therefore, falls down.

Likewise, the quality is affected by manuring, seed piece and spacing. Better grade of potato is obtained by large doses of manure, planting small seed pieces and greater distance between hills.

The cause, in effect, is precisely the same in all the three cases, *viz.*, a provision of better nutrition to the developing tuber. Heavy manuring provides it through increased available nutrients; small seed piece through decreased competition within the hills and large spacing through decreased competition between the hills.

TABLE VI

Average yield per hill in gm. under the treatment combinations of size of seed piece, spacing between hills and levels of manuring (1945-46)

	S_1			S_2			S_3		
	W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
M_1 ..	19·65	22·04	24·25	28·22	52·20	75·36	38·03	51·86	72·05
M_2 ..	41·37	54·40	56·78	82·72	132·52	138·52	85·95	133·37	139·22
M_3 ..	96·26	103·38	103·47	134·00	139·46	148·43	135·02	163·28	161·37

S. E. of a mean = 5·30 C. D. at 5% = 15·03

S. E. of a difference of means = 7·49 C. D. at 1% = 20·03

Interactions.—But plant growth and the consequent yield is a result of a number of factors and their influences have such complex combinations that it is of little practical value to know the absolute optimum of these factors; and it is in fact their group relation and interaction that is of greater consequence in actual crop production.

Results of the interaction between the three factors show that it is possible to get over in many cases the depreciating effect of one factor by changing one or both of the remaining two variables. Thus the 5 gm. seed piece which gives a significantly low yield compared to 10 and 20 gm. under the low manuring records but smaller differences when the manuring level is raised to M_3. A similar effect is noticed in other attributes also by introducing a change in one or more variables. Under such conditions, the possibility of using small seed pieces and adopting low seed rates, at least till normal price returns, in association with suitable adjustments in the other two components of manuring and spacing seems quite practicable.

CONCLUSIONS

1. Yield increases with more manure, larger size of seed piece and closer spacing between hills. Maximum increase is noticed under manuring factor.

2. Size of seed piece affects the yield but the degree of effectiveness varies with the level of manuring. At high manuring size of seed piece proves less effective and the difference in yield from 5, 10 and 20 gm. seed piece is relatively small.

3. As regards planting distance, a spacing of 8 inches is more suitable at low and medium manuring and with high manuring, close planting gives more yield.

4. Size of potato improves by heavy application of manure, using small seed pieces in comparison to large sets and adopting wider spacing between hills.

5. It is concluded that potato growing is not profitable under conditions of poor manuring. When manuring is moderate, the use of 10 gm. set sown 8 inches apart is more desirable and with heavy manuring 5 gm. seed piece sown 4 inches apart proves more useful.

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STUDIES ON FISH SPOILAGE

A comparative study of certain analytical methods of the estimation of Total Volatile base and Trimethylamine in Indian fish

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Introduction

Biochemical and bacteriological studies on fish spoilage are engaging the constant attention of various scientific workers in fish technology. Various chemical tests and their measurements like Indole (Clough, 1922), Hydrogen sulphide (Weaver, 1927), total volatile base (Glassmann and Rochwarger, 1929; (Beatty and Gibbons, 1937), Trimethylamine (Beatty and Gibbons, 1937), Tyrosine (Bradley and Bailey, 1940), Dimethylamine (Shewan, 1942), Histamine (Geiger, 1944), pH (Dyer *et. al.*, 1944) and total volatile acids (Sigurdsson, 1947) have been suggested to follow the decomposition of the fish muscle. The literature on the subject has been critically reviewed by Beatty and Gibbons (1937), Notevarp, Hansen and Karlsen (1942), Hansen and Bakken (1947), Ronald and Jakobsen (1947) and Sigurdsson (1947).

We have chosen the total volatile base and Trimethylamine investigations to follow the course of spoilage of fish muscle and present herein the method most suited for such estimations.

It has been conclusively proved by other workers that:—

(*a*) Trimethylamine Oxide is found in most of the marine organisms and is the precursor of trimethylamine—(Beatty and Gibbons, 1937 and 1939; Brocklesby and Riddel 1937; Beatty, 1938 and 1939; and Tarr, 1939) and

(*b*) that the estimation of trimethylamine in a marine fish is a reliable test for ascertaining the degree of freshness of fish—(Shewan, 1937; Beatty, 1939 and Notevarp, *et al.*, 1942).

Our aim in this preliminary communication is to bring out the relative merits and demerits of some of the methods available for the estimation of total volatile base and trimethylamine and to choose the best suited for our line of work.

Materials and Method of Work

Fish muscle from Mackerels (*Rastrelliger kanagurta*) landed at the Kozhikode (Calicut) beach was utilised for the experiments. The skin, bones, head, fins, intestine, etc., of mackerels were removed, and samples of thoroughly minced flesh alone were taken for extraction.

Three different extracts were obtained as outlined below on the minced sample and estimation for total volatile basic Nitrogen and trimethylamine nitrogen on each extract were carried out by the methods of estimation given below.

Extracts

(i) *Ethanol extract.*—To about 20 gm. of the minced sample in a mortar, added 30 ml. of 96% Ethanol in lots while stirring. Transferred the contents to a 100 ml. volumetric flask, and made up to mark with 96% Ethanol. Mixed well, filtered and aliquots of filtrate taken for estimation.

(ii) *Dry Crystal extract.*—To about 100 gms. of the sample 4 gms. of dry crystals of trichloroacetic acid were added and thoroughly mixed. After about 10 minutes the muscle juice was squeezed out by a small hand press, filtered and filtrate used for estimation.

(iii) *Trichloroacetic Acid solution—Water Extract.*—To about 20 gms. (accurately weighted) of the sample in a mortar, an equal quantity of water and twice the amount of 10% Trichloroacetic acid solution were added and mixed well. The homogenised substance was transferred to a volumetric flask and made up to mark with the above mixture. The contents of the flask were filtered and aliquots of the filtrate removed for analysis.

Estimation

(i) *Distillation procedure.*—To an aliquot of the extract, excess of pottassium carbonate (saturated solution) was added, and the total volatile base steam-distilled for 10 to 12 minutes under reduced pressure into excess of N/70 Sulphuric acid. The excess acid was back titrated with alkali of the same strength and the total volatile basic nitrogen calculated.

(ii) *Conway microdiffusion procedure.*—The method of estimation of total volatile base and trimethylamine in the extracts was the same as described by Beatty and Gibbons (1937).

(iii) Distillation procedure on the muscle direct.

Besides the above, steam distillation under reduced pressure directly on muscle taking 2 to 5 gms of the substance and using borax as the alkaliniser has been done for estimating the total volatile base only.

The results obtained on the various samples are tabulated below in the two tables.

TABLE I

The total volatile basic nitrogen values in mgm% on Mackerel

Sample No.	On muscle direct by steam distillation under reduced pressure	96% Ethanol extract		Dry Trichlor acid crystal extract		Acid-water extract	
		By distillation	By conway procedure	By distillation	By conway procedure	By distillation	By conway procedure
1	27·33	25·13	25·00	..	29·44	32·80	29·00
2	36·78	36·75	31·50	34·10	32·64	29·30	26·75
3	27·66	28·64	25·67	..	27·52	31·20	..
4	22·45	23·00	22·00	27·00	27·20	30·80	36·00
5	25·94	25·00	..	26·40	24·90	28·61	26·00
6	23·10	23·14	24·20	25·80	24·00	..	..
7	34·01	34·42	34·25	40·78	40·00	50·05	53·50
8	28·06	29·35	28·59	35·99	34·08	39·22	38·50
9	28·57	27·06	25·44	30·69	30·56	30·26	32·58

TABLE II

Trimethylamine nitrogen values in mgm% on Mackerel

Sample No.	96% Ethanol extract		Dry Trichlor acid crystal extract		Acid-water extract	
	By distillation procedure	By conway procedure	By distillation procedure	By conway procedure	By distillation procedure	By conway procedure
1	9·25	..	7·80	4·80	8·20	5·10
2	8·45	7·69	..	5·50	6·20	6·00
3	4·00	3·00	..	2·40	3·80	2·10
4	5·65	5·00	4·00	2·80	9·08	6·00
5	4·50	3·00	4·16	3·36	5·68	3·50
6	1·73	0·86	1·22	0·52	2·52	1·13
7	11·16	8·50	14·08	8·56	17·50	15·00
8	13·22	7·93	10·96	7·52	12·17	10·27
9	4·80	3·31	6·36	5·08	6·94	3·28

DISCUSSION

Table I gives the total volatile basic nitrogen values in mgm% and Table II gives the Trimethylamine Nitrogen values in mgm% (hereinafter called Triamine values for convenience) on the three extracts.

The study was confined only to the determination of the efficacy of the different methods of extracts and analysis, and so the analytical values cannot be expected to be the same for the different sample numbers, since

they were obtained on various dates and irrespective of the time lag after the death of fish.

Taking the total volatile base value determined by the steam distillation procedure under reduced pressure on the minced muscle direct, as the standard, we have compared the values obtained on the other extracts. The direct muscle determination value has been taken as our standard in conformity with conclusions of Beatty and Gibbons (1937) and Hansen and Bakken (1947). It will be seen that the values of the 96% Ethanol extract by both the methods of estimation are in good agreement with the values of direct estimation whereas the values of the dry crystal extract are not comparable to the same extent. The values of the acid water extract are generally high and bear no comparison even among the two methods. So, it can be concluded that for the determination of volatile base on fish muscle, the 96% ethanol extract is preferred, the method of estimation being the distillation procedure.

On turning to Table II which gives the triamine values, it will be seen that the acid-water extract values are again not comparable with any others. Only the values of the ethanol extract and the dry crystal extract are in agreement—the agreement being especially close when the values are compared with the same method for the two extracts, *i.e.*, the distillation procedure value on the ethanol extract being comparable to that obtained on the dry crystal extract by the same procedure, and similarly for the Conway procedure values.

From the above it will be seen that the ethanol extract is the most suitable for the total volatile base and triamine value estimations. The procedure to be adopted for the estimation of total volatile base on the extract, as we have already seen, is by distillation under reduced pressure. It follows naturally that the triamine also should be estimated on the same extract by the same procedure to avoid errors in sampling and in order to enable easy correlation between total volatile base and triamine values. We would like to point out at this stage that in studies on fish spoilage, the estimation of trimethylamine oxide is also important to follow its reduction by spoilage bacteria to trimethylamine, and for this an extract has to be obtained, reduced with a suitable chemical agent like Dewarda's alloy or titanium trichloride. Distillation is the only procedure left since the Conway technique does not lend itself to easy adaptation due to the unwieldy volume of the final reduced extract. This again stresses the importance of the distillation procedure as the most suitable for the trimethylamine estimation.

Summing up, for studies on fish spoilage involving estimation of total volatile base, trimethylamine and its oxide, the estimations can preferably be made by steam distillation under reduced pressure, on a suitable aliquot of the 96% ethanol extract.

Summary

In this communication, we have indicated the lines on which future analysis for estimation of disintegration products in fish muscle will be made by us with a view to evaluate the various stages of fish spoilage.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE SYSTEMATIC POSITION OF THE SPHENOCLEACEÆ

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(Communicated by Dr. P. Maheshwari, F.A.SC.)

Engler (1897) placed the genus *Sphenoclea* Gaertn., so far known to include only two species, under the Campanulaceæ. Hutchinson (1926) divided this family into the Campanulaceæ and Lobeliaceæ on the basis of the actinomorphic or zygomorphic nature of the flowers, and the extent to which the anthers are free or laterally coherent. Of the two species so far known, *Sphenoclea zeylanica* Gaertn. is pantropical and is found in South India in marshy regions. Much interest has been aroused in this genus following the recent work of Airy Shaw (1948) who has raised it to the rank of a family. According to him it shows no relationship with the Campanulaceæ under which it was included till recently, but resembles *Phytolacca* in habit and anatomy. In certain other features, the family Sphenocleaceæ is said to resemble the Primulaceæ, and Airy Shaw recommends a place for it between the Phytolaccaceæ and Primulaceæ.

However, a comparison of the embryological and anatomical features of *Sphenoclea* with those of the Phytolaccaceæ reveals important differences which are tabulated on the next page (Table I).

Some of the differences in Table I are so fundamental that any close relationship between these two families seems to be out of question.

Turning now to the family Primulaceæ, we find that here also the differences are more pronounced than the resemblances (Table II).

Coming finally to the Campanulaceæ in which *Sphenoclea* has been placed by most workers, Table III summarises the recent work on this genus (Kausik and Subramanyam, 1946 *b*) and several other members of the Campanulaceæ (Rosén, 1932, 1949; Souéges, 1936, 1938; Kausik and Subramanyam, 1947 *a*; Subramanyam, 1947, 1948; Crété, 1948) and Lobeliaceæ (Rosén, 1932, 1949; Kausik, 1935, 1938; Crété, 1938, 1939; Hewitt, 1939; Maheshwari, 1944; Kausik and Subramanyam, 1945 *a* and *b*; 1946 *a* and *c*; 1947 *b*; and Subramanyam, 1950).

TABLE I

Family ..	*Sphenoclea zeylanica* (D'almeida, 1934; Kausik and Subramanyam, 1946 *b*; Solereder, 1908)	Phytolaccaceæ (Joshi, 1936; and Rama Rao, 1936; and Kajale, 1944; Solereder, 1908)
Aerating tissue in cortex of stem	Present	Absent
Oxalate of lime ..	Large clustered crystals present	Usually does not occur in the form of solitary or clustered crystals, but in the form of raphides, styloids, raphide-sacs, sphaerites, etc.
Anomalous secondary growth	Absent	Present
Ovule ..	Anatropous unitegmic	Ana-campylotropous, bitegmic
Nucellus ..	Very thin and ephemeral	Massive; present even in old seeds
Nucellar epidermis	Completely destroyed by the developing embryo sac	Produces a secondary parietal tissue
Endothelium ..	Present and quite conspicuous	Absent
Endosperm ..	Cellular, with well developed haustoria	Free nuclear, haustoria absent
Embryo ..	Onagrad type[1]	Caryophyllad type[2]
Mature embryo..	Straight	Curved

TABLE II

Family ..	*Sphenoclea zeylanica* (Kausik and Subramanyam, 1946 *b*; Solereder, 1908)	Primulaceæ (Dahlgren, 1916; Schnarf, 1929, 1931; Solereder, 1908; Souèges, 1937)
Oxalate of lime..	Large clustered crystals present in leaves	Absent in vegetative organs
Anomalous secondary growth	Absent	Present in some genera
Placentation ..	Axile	Free central
Ovule ..	Unitegmic	Bitegmic
Endosperm ..	Cellular, haustoria well developed	Free nuclear, becomes cellular in later stages; haustoria absent
Endosperm in mature seed	Scanty or none	Fleshy or horny
Embryo ..	Onagrad type; fairly large	Caryophyllad or Solanad type[3]; rather small

[1] In the Onagrad type of embryo (Johansen, 1945) the first wall in the terminal cell of the two-celled proembryo is vertical, and the basal cell has little or no part in the construction of the embryo proper.

[2] In the Caryophyllad type (Johansen, 1945), the first wall in the terminal wall of the two-celled proembryo is transverse, and the basal cell becomes converted into a large suspensor cell. The terminal cell produces the embryo and also contributes to the suspensor.

[3] In the Solanad type (Johansen, 1945) the basal cell of the two-celled proembryo plays no essential part in the construction of the embryo and produces a suspensor of two or three cells. The first division of the terminal cell is transverse.

TABLE III

Family ..	*Sphenoclea zeylanica*	Campanulaceæ
Wall of the anther	Three layers outside tapetum	Same
Tapetum ..	Glandular type; cells binucleate	Same
Arrangement of microspores	Tetrahedral, rarely isobilateral	Same
Shedding stage of pollen	Trinucleate; exine smooth with 3 germ pores; starch grains absent	Trinucleate; exine smooth or worty with 3 or 4 germ pores; starch grains sometimes present
Ovule ..	Indefinite in number, anatropous, unitegmic, tenuinucellate	Same
Endothelium ..	Present	Present
Embryo sac ..	Polygonum type; mature embryo sac long and tapering at the ends	Same
Synergids ..	Not beak-shaped	Beak-shaped
Antipodals ..	Three cells	Same
Endosperm ..	Cellular, follows a slight modification[5] of the Scutellaria type of Schnarf (1931)	Cellular, follows the Scutellaria,[4] or Codonopsis,[6] or Phyteuma,[7] or rarely Isotoma type[8]
Endosperm haustoria	Micropylar haustorium 4-celled; chalazal haustorium also 4-celled	Micropylar haustorium 2-celled; chalazal haustorium 2-celled or 1-celled
Endosperm in mature seed	Scanty or absent	Abundant
Embryo ..	Onagrad type	Solanad type
Structure of the seed coat	Inner walls of epidermal cells conspicuously thickened into radial spine-like outgrowths	Outer and later walls of the epidermis thickened; each cell has communication with neighbouring cells by means of canals, which traverse the entire thickness of the cell wall

[4] In the Scutellaria type of endosperm (Schnarf, 1931), after the 8-celled stage, the two uppermost cells and the two basal cells develop into the micropylar and chalazal haustoria respectively (Fig. 1–A).

[5] In *Sphenoclea zeylanica* (Kausik and Subramanyam, 1946*b*), there is a slight modification of the Scutellaria type. After the formation of the 8-celled endosperm, further vertical walls are laid down at right angles to the first resulting in a 16-celled endosperm arranged in four tiers of four cells each. Now the uppermost tier and lowermost tier develop into the 4-celled micropylar and chalazal haustoria respectively (Fig. 1–B).

[6] The Codonopsis type (Rosén, 1932), resembles the Scutellaria type of Schnarf in early stages, but after the formation of the 8-celled endosperm, one or at least two more transverse divisions are laid down in the basal tier of cells, after which alone the 2-celled chalazal endosperm haustorium is delimited (Fig. 1–C).

[7] In the Phyteuma type (Rosén, 1932), after the formation of the primary micropylar and chalazal chamber, a vertical wall is laid down in the former and a transverse wall in the latter. The result is that while the micropylar haustorium is 2-celled the chalazal is 1-celled (Fig. 1–D).

[8] The Isotoma type (Kausik and Subramanyam, 1945*a*), although apparently similar to the Phyteuma type, shows some differences in the initial stages of endosperm development (Fig. 1–E).

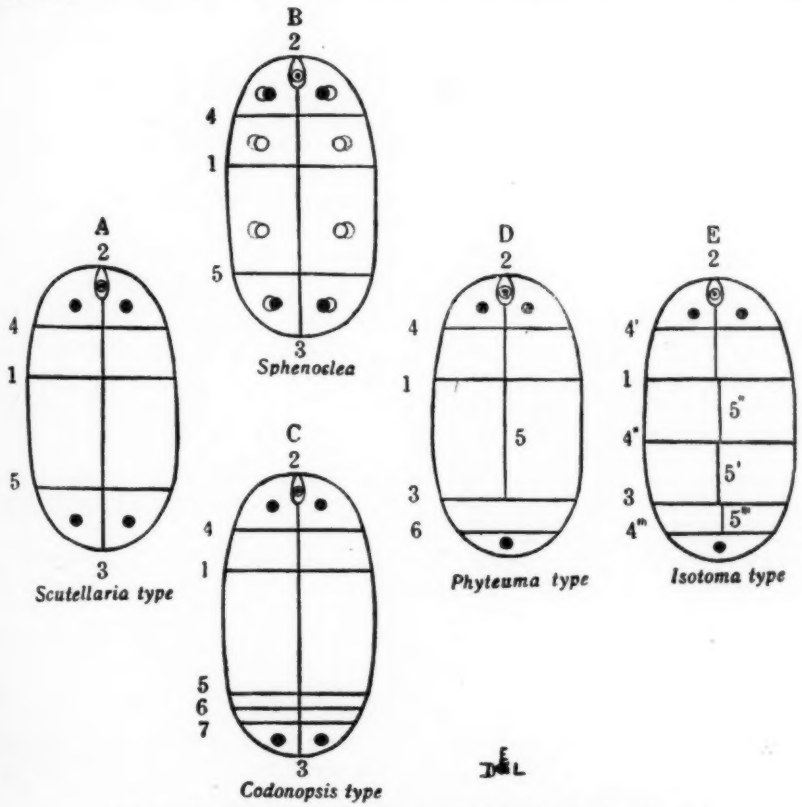

FIG. 1. Types of Endosperm Development in the Campanulaceæ.
The numbers indicate the sequence of wall formation.

A study of the data tabulated above suggests that although *Sphenoclea* has many points of resemblance with the Campanulaceæ, it has some distinctive features of its own which lend support to the creation of a separate family, the Sphenocleaceæ. However, this family must remain a part of the order Campanulatæ for there is no evidence of its being related to either the Phytolaccaceæ or the Primulaceæ. Some of the anatomical characters like oxalate of lime, anomalous secondary growth and embryological characters such as unitegmic tenuinucellate ovules, massive placenta, endothelium, cellular endosperm with prominent endosperm haustoria and the Solanad or the Onagrad type of development, are features which need to be recognised as fundamentally different from those of any known Phytolaccaceæ or Primulaceæ.

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SOIL CONDITIONS AND WILT DISEASES IN PLANTS WITH SPECIAL REFERENCE TO *FUSARIUM VASINFECTUM* ON COTTON*

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Introduction

Considerable work has been done in the United States, Egypt, and in India on the problem of wilt in cotton caused by *Fusarium vasinfectum* (Atkinson, 1892; Orton, 1900, 1908; Fulton, 1907; Hibbard, 1910; Gilbert, 1914; Neal, 1927; Taubenhaus, *et al.*, 1928; Fahmi, 1927; Fikry, 1932; Butler, 1912–13, 1926; Ajrekar and Bal, 1921; Dastur, 1924, 1931; Ajrekar, 1926; Bal, 1926; Kulkarni and Mundkur, 1931; Kulkarni, 1934, 1941; Mitra and Kheswalla, 1935). The economic implications of this disease are well known, and they assume greater importance in the light of the new records for this disease in different cotton-growing countries. It is fast becoming a world problem and, although much work has already been done on the etiology of the disease and morphology and physiology of the causal fungus, the question of control remains to be solved. Soil amendments have been tried by a number of workers, but with no favourable results (Fulton, 1907; Hibbard, 1910; Gilbert, 1914; Lewis, 1911; Neal, 1927), though some measure of control has been reported through potash fertilizers in the United States (Young, 1940; Young and Tharp, 1939, 1941). The application of the results obtained by these American workers offers no solution to the problem in India, since the relation of the disease to soil conditions is so different in the two countries (Fikry, 1932; Neal, 1927; Kulkarni, 1934; Mundkur, 1936). In general, both in the United States and Egypt, and also in India, many workers now look to selection and breeding for resistance as the only solution for the control of the disease (Kulkarni, 1934; Uppal, Kulkarni and Ranadive, 1940; Fahmi, 1942). However, as pointed out by Brooks (1935), breeding for resistance has limitations in that parasitism and even virulence are often gradually acquired by parasitic fungi, the evolution of parasitic strains being the result of mutation, and the building up

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of resistant varieties is a time-consuming and laborious process and not worth while when other comparatively simple methods in cultivation, soil management and sanitation would constitute more efficacious treatment for control. It is now recognized that in the case of soil-borne diseases at least control may be achieved through modification of soil conditions, the literature on which subject has been reviewed by Garrett (1944). In the case of vascular parasites, however, Garrett (1947) has pointed out that modification of soil conditions may not be of much use in controlling the disease on the crop. In such cases the only method of control would be to expedite the elimination of the causal fungus from the " wilt-sick " soil through modification of soil conditions. The real solution perhaps lies in the application of the principles of microbial interaction and antibiosis in soils which have now become fundamental to the study of soil microbiology (Waksman, 1947). Indeed, the author's preliminary work (Subramanian, 1946 *a*) has already shown that *Fusarium vasinfectum* is highly sensitive to microbial antagonism and does not survive for long in soils under the operation of this factor. Since the publication of these results, the author had the opportunity to continue his investigations on the behaviour of *Fusarium vasinfectum* in the soil, with particular reference to growth, survival and saprophytic activity. The results of these investigations, which were taken up at the kind suggestion of Professor T. S. Sadasivan, Director of the Laboratory, are presented below.

Materials and Methods

I. General

Standard mycological technique was employed throughout the investigation (Rawlins, 1933; Fred and Waksman, 1928).

Fusarium strains used.—Two strains of the cotton wilt *Fusarium* have been used constantly during the course of this investigation. They were: (i) *Fusarium vasinfectum*, a strain of the fungus obtained from the Government Mycologist, Coimbatore, S. India; and (ii) a strain of the *Fusarium* isolated from wilted cotton plants collected from Udamalpet (Coimbatore District, South India) " wilt-sick " fields. These are referred to respectively as strains I and II. Both the strains proved pathogenic to cotton plants under laboratory conditions. Single spore cultures of these fungi were obtained by the plate dilution method (Smith, 1946).

Soils used: *Collection.*—"Wilt-sick" black cotton soil from the Udamalpet infected fields was collected by the usual methods. Where necessary, periodical collections of soil samples were made. This soil is referred to as Udamalpet soil and was usually air-dried after collection.

Two types of soil were used: (1) Udamalpet soil. pH about 8; water-holding capacity 70–80; typical heavy and clayey black cotton soil; and (2) Compost soil prepared by mixing stable manure, unwashed sand and red earth in the 2:2:1 ratio; pH about 6·5, water-holding capacity 33.

The pH of soils was determined by Beckman's pH meter and water-holding capacity by the Keen-Raczkowski method (Keen and Raczkowski, 1921). All soils were air-dried and passed through a sieve with meshes 1/18″ square before use.

Soil moisture.—Soil moisture was initially adjusted at desired levels on the basis of percentage water-holding capacity. Calculated amounts of sterilised distilled water were added to known weights of soil in suitable containers. Where earthenware pots were used allowance was made for the absorption of water by the pots. Maintenance of moisture at the various levels was by calculation of the average loss per container due to evaporation and supplying the calculated amount periodically to the soils. Where greater accuracy in regard to percentage moisture was needed, glass containers were used for the soils.

Incubation.—Soils were usually incubated at room temperature which ranged from 28–32° C.

Sterilisation.—Sterilisation of pots, containers and soil was effected by autoclaving at 20 pounds pressure for 2 hours.

Soil inoculation.—Inoculation of soil in pathogenicity tests was done by incorporating into soil inoculum of *Fusarium vasinfectum* grown in Shive's medium.

Media.—The following media, prepared by the usual methods, were used: (i) Horne and Mitter's agar (Rawlins, 1933) using KH_2PO_4 in place of K_3PO_4 and soluble starch instead of potato starch; (ii) potato dextrose agar (Fred and Waksman, 1928); (iii) Shive's agar prepared according to McLean and Cook (1941), but adding 1·0% soluble starch, 0·2% dextrose and 2% agar; and (iv) Barnes' agar (Rawlins, 1933).

II. Techniques

1. *To study the growth and survival of* Fusarium vasinfectum *in bare soils*

(*a*) *Rossi-Cholodny slide technique.*—The modified technique used here has already been described in detail in a previous publication (Subramanian, 1946 *a*). Shive's agar, instead of Horne and Mitter's, was used for coating the Cholodny slides. The degree of decomposition of the *Fusarium* mycelium, etc., was quantitatively expressed by recording the presence or absence of

hyphæ, etc., in fifty microscopic fields. Each microscopic field corresponded in area to that obtained by the use of a × 40 objective and × 10 eyepiece. The presence of (*a*) *Fusarium* hyphæ, (*b*) *Fusarium* conidia, and (*c*) *Fusarium* chlamydospores were recorded for each of the 50 fields examined on each slide. The frequency of occurrence of *Fusarium*, expressed as percentage of fields examined under the microscope, was taken to indicate the extent of decomposition of *Fusarium*.

(*b*) *Plating method.*—Equal amounts of *Fusarium vasinfectum* soil (inoculum) were taken in earthenware pots, and equal amounts of unsterilised soil were placed over the soil inoculum as an upper layer. The degree of survival and the spread of *Fusarium* were both periodically estimated by a quantitative analysis of *Fusarium* present in the lower and upper layers respectively of the soil in pots. Representative samples of soil from the upper and the lower layers of each pot were plated out on Shive's agar using a final dilution of 1/10,000 and employing the usual methods (Brierley, *et al.*, 1927). The *Fusarium* colonies alone, if any, appearing on the plates were counted and the *Fusarium* content was expressed in thousands per gram of air-dry soil.

(*c*) *Colonisation method.*—Udamalpet soil (collected in February 1945) was subjected to certain definite treatments (these treatments are enumerated under Experiments VIII and IX) for a period of 16 and 32 weeks at the end of which the soil was air-dried. Cotton root bits of uniform size were then buried in the variously treated soils and incubated for a period of 8 weeks in the soil (50 per cent. moisture), after which the root bits were plated out and the number of bits giving *Fusarium* determined. The number of root bits giving *Fusarium* was taken to be an index of survival of the fungus in the soil. The methods followed in the burial and plating out of root bits have been described in detail elsewhere (Subramanian, 1946 *b*). Shive's agar was used for the final root platings. Using standard methods (Brierley, *et al.*, 1927), microbial numbers in each treatment were determined by plate counts, samples from the variously treated soils being plated out at intervals of four weeks for sixteen weeks. Horne and Mitter's agar was used for plating.

2. *To study the saprophytic activity of the fungus*

"*Root burial*" *technique.*—The technique which is a modification of the one originally devised by Sadasivan (1939) in his work on colonisation of wheat straws by *Fusarium culmorum* and other soil fungi, has been described in detail by the author (Subramanian, 1946 *b*). Shive's agar, instead of Horne and Mitter's, was used for the root platings. Throughout this

work *Fusaria* colonising root bits will be referred to only as *Fusarium* spp., since work on pathogenicity, morphology and physiology of these isolates is in progress and will form part of a further communication.

Experimental

Growth and survival of Fusarium vasinfectum *in bare soil*

Experiment I

In this experiment the effect of moisture and temperature on growth and survival was studied. Cholodny's technique was used. The moisture percentage of the soil was adjusted to and maintained at the following levels: 30, 50, 70, 90 and 100 per cent. Air-dry soil served as control. One set of soils was incubated at room temperature (29–31° C.) and another in the refrigerator (temperature 11–14° C.). The slides were removed and examined at the end of 2, 5, 9 and 14 days. The results are presented in Table I.

Conclusions.—(i) In all cases *Fusarium* was attacked and decomposed by antagonistic micro-organisms, especially bacteria.

(ii) Regarding the effect of moisture on decomposition of *Fusarium* hyphæ, at both temperatures, decomposition was minimum in the air-dry soil and maximum at 90 per cent. moisture. The slight decomposition shown by the slides buried in the air-dry soil is attributable to the moisture present on the slides themselves, since these were not air-dried before burial. Moisture rather than temperature was a limiting factor for decomposition of the hyphæ. The rate and extent of decomposition at both temperatures increased progressively from the air-dry treatment with addition of moisture up to 90 per cent. followed by a fall in the rate and extent of decomposition. At 100 per cent. moisture level decomposition was somewhat less than at 90 per cent.

(iii) The extent and rate of decomposition was lower at 11–14° C. than at 29–31° C. at both 30 and 50 per cent. moisture levels.

(iv) Conidial decomposition was less at 11–14° C. than at 29–31° C.

(v) At 29–31° C. there was no decomposition of chlamydospores in the dry soil; there was slight decomposition at 30–70 per cent. moisture levels. The decomposition was considerable at 90 per cent. moisture.

(vi) At 11–14° C. practically no decomposition of the chlamydospores could be observed.

TABLE I

Percentage fields with Fusarium *hyphæ, conidia and chlamydospores on Cholodny slides*

Incubation in days	Temperature of incubation in °C.	Moisture level																	
		Air-dry			30%			50%			70%			90%			100%		
		Hy.	Co.	Chl.	Hy.	Co.	Chl.	Hy.	Co.	Chl,	Hy.	Co.	Chl.	Hy.	Co.	Chl.	Hy.	Co.	Chl.
2	29–31	98	38	66	88	36	58	72	36	58	68	36	60	24	None seen	38	28	None seen	None seen
	11–14	98	64	66	98	64	70	98	62	68	94	64	66	50	do	68	58	do	70
5	29–31	98	36	64	76	34	60	66	32	58	64	34	60	—*	—*	—*	26	dc	None seen
	11–14	96	64	66	98	60	68	96	58	64	68	60	66	46	None seen	66	56	do	66
9	29–31	96	36	64	68	22	58	58	14	58	44	12	58	18	do	None seen	22	do	None seen
	11–14	98	64	72	94	54	66	90	50	68	60	48	66	44	do	66	48	do	72
14	29–31	94	36	66	34	14	58	24	8	58	24	2	58	16	do	None seen	16	do	None seen
	11–14	98	64	66	94	44	66	78	46	68	56	44	66	38	do	64	46	do	70

Hy.= Hyphæ ; Co.= Conidia; Chl.= Chlamydospores. —* Slides lost.

Experiment II

In the present experiment the Rossi-Cholodny technique was employed to study the interaction of soil organisms in unsterilised and sterilised recontaminated soil with reference to the behaviour of *Fusarium vasinfectum*. The experiment consisted of the following treatments:—

Treatment—

A. Sterilised and recontaminated with unsterilised soil;

B. Sterilised and recontaminated with 'aerial plankton' for five minutes;

C. Sterilised and recontaminated with 'aerial plankton' for one hour;

and D. Unsterilised.

Recontamination of sterilised soil was effected under aseptic conditions by the addition of one gram of moist unsterilised soil. Recontamination by the 'aerial plankton' was accomplished by exposing such sterilised soils for different periods of exposure. The results are presented in Table II.

TABLE II

Percentage fields with Fusarium *hyphæ on Cholodny's slides buried in soils variously treated*

Days of incubation	Treatments			
	Sterilised and recontaminated with soil	Sterilised and recontaminated with "aerial plankton" for		Unsterilised
		5 min.	1 hr.	
1	100	100	100	98
2	95	96	94	78
3	85	82	80	68
5	82	70	70	63
10	70	46	45	51
17	39	35	34	43
26	14	10	10	18
35	0	0	0	0

It was seen that the decomposition of *Fusarium* in the soil was markedly affected by two factors pertaining to the microflora bringing about the decomposition. Firstly, it could be generally stated that on the *state of equilibrium* of the microflora depends the rate of decomposition of the *Fusarium* inoculum. This factor was less in the unsterilised soil with its natural and comparatively stable microflora than in the recontaminated sterilised

soil with an unstable microflora. Secondly, the *nature* or *specific composition* of the microflora also affects the rate of decomposition. Treatments A, B and C simulated each other in having microfloras fast developing and progressing towards an equilibrium in the virgin sterilised soil. The specific composition of the flora in A, on the one hand, and in B and C, on the other, however, was different. The period for which the treatments B and C were subjected to recontamination evidently did not matter in so far as the equilibrium and nature of the flora developing in them were not different.

Experiment III

The effect of microflora on pathogenicity of *Fusarium vasinfectum* on cotton (KI variety) was next studied using unsterilised soil and sand, and sterilised soil and sand subject to recontamination by an invading flora. The results are presented in Table III.

TABLE III

Pathogenicity of Fusarium vasinfectum *on cotton in unsterilised soil and sand, and sterilised recontaminated soil and sand*

Treatments	Total No. of plants used for inoculation tests	Number of plants wilted at the end of			
		5 days	8 days	10 days	12 days
Soil sterilised (recontaminated) ..	43	3	2	19	19
Soil unsterilised ..	32	None	5	24	3
Sand sterilised (recontaminated) ..	45	11	7	5	22
Sand unsterilised ..	30	None	20	10	..

It will be seen from the results that infection occurred earlier in the sterilised treatments; later, however, the progress of wilt was more rapid in the unsterilised treatments than in the sterilised. This is attributable to the fact that the microflora invading sterilised soil and sand had a more depressing effect on *Fusarium* pathogenicity than the indigenous flora in unsterilised soil. Periodical determination of microbial numbers in the treatments (see Table IV) indicated that, though initially the microbial numbers (fungi and bacteria) in sterilised soil and sand were lower than those in unsterilised treatments, these later on became numerically greater than in the parallel unsterilised treatments. This finding confirms the

TABLE IV

Microbial numbers in thousands per gm. in sterilised and unsterilised treatments periodically

Treatment	1 day	9 days	14 days
Soil sterilised ..	120	16480	17260
Soil unsterilised ..	7040	11920	14760
Sand sterilised ..	40	380	3500
Sand unsterilised ..	320	320	3200

observations of a number of workers on the depressing effect of recontamination of sterilised soil on the pathogenicity of soil-borne parasites (Broadfoot, 1933; Sanford, 1941; Roberts, 1943; Ludwig and Henry, 1943).

Experiment IV

The results of the previous experiment showed that the invading microflora had a profound inhibiting effect on pathogenicity. It was, therefore, felt that observations on the interaction between *Fusarium vasinfectum* mycelium and the normal microflora *in situ* by using the Cholodny slide technique would elucidate the nature and mode of the antagonistic action. A large number of stained as well as unstained fresh slides (soon after removal from the soil) were examined. Representative photomicrographs showing the various stages in the decomposition of the *Fusarium* are reproduced in Plates IV and V.

The various stages in the decompostion of *Fusarium* are described below. Living hyphæ stained well but on partial or complete decomposition did not do so and this is in keeping with the observations of Conn (1932) and Starkey (1938). The *modus operandi* of the bacteria in the process of fungal decomposition was one of intense crowding around the hyphæ impinging directly on the hyphal wall, resulting in the final break up of the hyphæ (Plate IV, Figs. 1-3). Observations to this end have been made by a number of workers (Starkey, 1938; Humfeld and Smith, 1932; Krassilnikov, 1936). In the preliminary stage of decomposition of the hyphæ the bacterial population had the tendency to assemble around hyphæ or spores as observed above but progressive decomposition of the hyphæ was marked by linear vacant spaces around which dense masses of bacteria were noticed (Plate IV, Fig. 4). The final stage of decomposition was represented by an outline of the fungal hyphæ and the bacteria being scattered all over the slide (Plate IV, Fig. 5).

Conidia of *Fusarium* also underwent decomposition by bacteria but appeared to be more resistant to bacterial attack than vegetative hyphæ as judged by the time taken to lose power of staining (Plate V, Fig. 8). As in the case of the hyphæ, when decomposition was complete, conidia-shaped vacant spaces were seen enveloped by bacteria and these signified the spaces originally occupied by both the micro and macro-conidia before they underwent decomposition (Plate IV, Fig. 6; Plate V, Fig. 7).

lamydospores of *Fusarium* underwent decomposition by bacteria in a similar manner, although they were more resistant to decomposition than either hyphæ or conidia (Plate V, Fig. 9). Healthy, undecomposed chlamydospores took up the stain well and where decomposition had proceeded considerably only a portion of the thick wall was stained, the contents being absent (Plate V, Figs. 10, 11). The author has come across certain stray cases of chlamydospores germinating *in situ* while the rest of the hypha bearing the chlamydospores was attacked. It must be finally stated that once the conidia or chlamydospores germinated, the promycelia were immediately attacked by the antagonistic bacteria (Plate V, Fig. 12), thus ruling out vegetative growth in the presence of bacterial antagonism.

Experiment V

Many workers have stressed the inhibitory effect of carbon dioxide on fungal growth (Brown, 1922; Fellows, 1928) and also the probable inhibition of fungal growth in soils due to the accumulation of microbial respi-

TABLE V

Growth in diameter (in millimetres) of Fusarium vasinfectum *and other soil fungi incubated in normal and carbon dioxide atmosphere*

Incubation in days	*F. vasinfectum* strain II		*Dactylaria* sp.		*Pythium aphanidermatum*		*Rhizoctonia bataticola*		*Trichoderma lignorum*	
	Normal atm.	CO_2 atm.	Normal atm.	CO_2 atm.	Normal atm.	CO_2 atm.	Normal atm.	CO_2 atm.	Normal atm.	CO_2 atm.
2	26	8	38	32	120	70	43	40	45	42
3	36	10	50	44	..	..	72	68	82	78
4	45	13	61	57	..	..	90	84	..	..
5	51	15	72	66	..	..	..	..	..	..
6	61	18	77	75	..	..	..	..	..	..
7	71	20	85	84	..	..	..	..	..	..

ratory carbon dioxide (Garrett, 1936; Blair, 1943). In view of these observations, a study of the effect of carbon dioxide on *Fusarium vasinfectum* and other soil fungi and its relation to growth of these in soils was made. The results are presented in Table V.

Table V shows that there is no definite relation between capacity for free growth in unsterilised soil and susceptibility to carbon dioxide as a growth-inhibiting factor. The growth of *Fusarium vasinfectum* and *Pythium aphanidermatum* was inhibited considerably while *Rhizoctonia bataticola*, *Dactylaria* sp. and *Trichoderma lignorum* did not show any marked inhibition of growth in the presence of carbon dioxide in pure culture. *Dactylaria* sp., *R. bataticola* and *P. aphanidermatum* unlike *F. vasinfectum* have the capacity to make free growth in unsterilised soils (Kovoor, 1947 unpublished; Sadasivan, 1946, unpublished; Subramanian, 1947 unpublished). Yet their behaviour in pure culture with regard to inhibition by carbon dioxide was different.

Experiment VI

To clarify the position further the problem was pursued using the Cholodny's technique: The experiment consisted of soil amendments with (*a*) 2 per cent. stable manure, and (*b*) 5 per cent. stable manure; and (*c*) untreated control. Two container jars of each treatment were incubated in carbon dioxide free atmosphere. The results obtained showed that (i) there was no appreciable difference in the extent of progress of decomposition between the treatments incubated in the carbon dioxide free atmosphere and parallel ones incubated in the normal atmosphere from which the carbon dioxide was not eliminated; (ii) the addition of manure accelerated decomposition in all cases even when the respiratory carbon dioxide produced by the micro-organisms was removed as soon as it was produced.

Experiment VII

In this experiment the growth and survival of *Fusarium vasinfectum* in artificially infected soil was studied employing the plating method already described (see under Methods): unsterilised soil was placed as an upper layer over a lower layer of a soil inoculum of the fungus, and the spread of the fungus into the upper layer and the survival of the same in the lower inoculum were tested by plating out samples from the upper and lower layers respectively. *Fusarium* colonies appeared only in the case of the lower layers of soil plated out, these being completely absent from the upper layers. The results presented in Table VI indicate the gradual disappearance and diminution in numbers of *F. vasinfectum* (strains I and II) in the soil, evidently due to the antagonism of the microflora of the upper layer of unsterilised soil. The complete absence of the fungus from the upper layers of soil confirms the

TABLE VI

Showing decrease in Fusarium vasinfectum *numbers (thousands per gm.) in soil at weekly intervals*

Organism	Time of incubation in soils in weeks					
	1	2	3	4	5	6
F. vasinfectum strain I ..	340	280	260	180	120	40
F. vasinfectum strain II..	400	320	240	200	100	60

observations already made regarding the inability of *Fusarium vasinfectum* to make any extensive growth in soils under ordinary conditions.

Experiment VIII

In this experiment the effect of moisture on survival of *Fusarium* in Udamalpet soil was studied using the 'colonisation method' (see under Methods). Soil moisture levels were maintained at 30, 50, 70, 90 and 100 per cent. of the water-holding capacity. The results are presented in Table VII and indicate the following:

TABLE VII

Survival of Fusarium *in Udamalpet "wilt-sick" soil*

Soil moisture %	No. of root bits colonised by *Fusarium* in soils incubated for 16 weeks	No. of root bits colonised by *Fusarium* in soils incubated for 32 weeks	Average microbial numbers (thousands per gm. of soil)
Air-dry	30	30	14200
30	16	12	94675
50	30	20	76286
70	28	20	42600
90	30	20	56325
100	28	26	58400

Total No. of root bits buried in each case was 30.

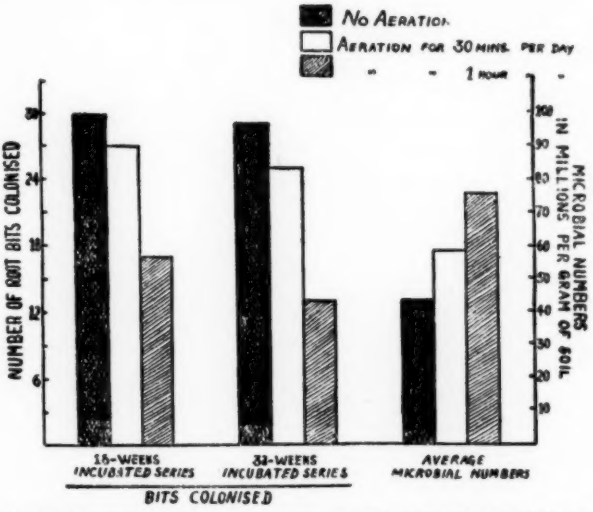
Conclusions.—(i) Irrespective of the period of incubation the maximum colonisation in air-dry soil was undiminished indicating the absence of microbial activity in the soil in the absence of moisture.

(ii) Where moisture was supplied to the soil, there was minimum survival at 30 per cent. moisture and maximum at 100 per cent. moisture.

(iii) Survival of *Fusarium* and microbial activity as indicated by microbial numbers (Table VII) bear an inverse correlation.

Experiment IX

The effect of aeration on *Fusarium* survival in Udamalpet soil was next studied, using the 'colonisation method'. The soil was aerated in glass containers under aseptic conditions using an air pump delivering 1,100 ml. of air per minute. One set of bottles was aerated for half an hour per day; another set was similarly aerated for one hour per day. A third set of bottles left without forced aeration, served as control. The results are given in Text-Fig. 1.


No Aeration
Aeration for 30 mins. per day
" - 1 hour "
NUMBER OF ROOT BITS COLONISED
MICROBIAL NUMBERS IN MILLIONS PER GRAM OF SOIL
18-WEEKS INCUBATED SERIES
33-WEEKS INCUBATED SERIES
AVERAGE MICROBIAL NUMBERS
BITS COLONISED

TEXT-FIG. 1. Effect of aeration on survival of *Fusarium* (indicated by the number of root bits colonised) and on microbial numbers in the Udamalpet "wilt-sick" soil.

The results indicate a close inverse correlation between degree of survival of *Fusarium* in the soil and microbial activity (as indicated by microbial numbers), the latter being enhanced by forced aeration.

Survival of Fusarium vasinfectum *in sterilised soil and sand*

Experiment X

Since *Fusarium vasinfectum* did not survive for long in unsterilised soil, it was considered important to study its survival in sterilised soil and sand.

It was expected that the fungus, if maintained in pure culture in the soil free from microbial antagonisms, might persist and remain viable in the soil for a considerable period of time. Compost soil and unwashed sand (50 per cent. moisture) in test-tubes were sterilised and inoculated with pure cultures of *F. vasinfectum*. The sand and soil cultures were plated out in potato dextrose agar at monthly intervals using the dilution method. The fungus was found to remain viable for a period exceeding twenty-four months.

The Saprophytic activity of Fusarium

Preliminary investigation (Subramanian, 1946 *b*) showed that *Fusarium* was a primary and dominant coloniser on cotton root bits buried in Udamalpet " wilt-sick " soil. Since colonisation of root bits by *Fusarium* would ensure its survival in soils by reducing its vulnerability to microbial attack, this saprophytic phase of the fungus on root bits had to be studied in detail.

Experiment XI

The capacity of *Fusarium* spp. to colonise substrata other than cotton roots was studied in this experiment. Plant parts of a number of plants were buried in soil and plated out, using the 'root burial' technique. The results are summarised in Table VIII.

TABLE VIII

Percentage colonisation of Fusarium spp. *on parts of various plants buried in Udamalpet " wilt-sick " soil*

Name of plant	Part of plant used for burial	Percentage colonization at the end of 4 weeks
Gossypium arboreum (cotton) ..	Root	100
,,	Stem	95
,,	Petiole	55
Hibiscus esculentus (bhindi or okra) ..	Root	100
,,	Stem	93
,,	Petiole	35
Vigna catjang (cowpea) ..	Root	53
,,	Stem	37
Cajanus cajan (red gram) ..	Root	53
,,	Stem	70
Euphorbia hirta ..	Root	100
Acalypha sp. ..	Root	90
Croton sparsiflorus .	Root	100
Cleome viscosa ..	Root	50

These results were supplemented by the isolation of *Fusarium* from the stubble and other plant residues collected from " wilt-sick " soils. Similarly, stubbles collected from the Udamalpet infected fields were also plated

out on potato dextrose agar. The highest figures for *Fusarium* colonisation were obtained for cotton roots, and somewhat similar figures for stubble of wheat (*Triticum vulgare*) and bengal gram (*Cicer arietinum*). These facts, brought out by a study of stubble collected from infected fields, have added to the importance of the demonstration of the saprophytic activity of *Fusarium* spp. under laboratory conditions.

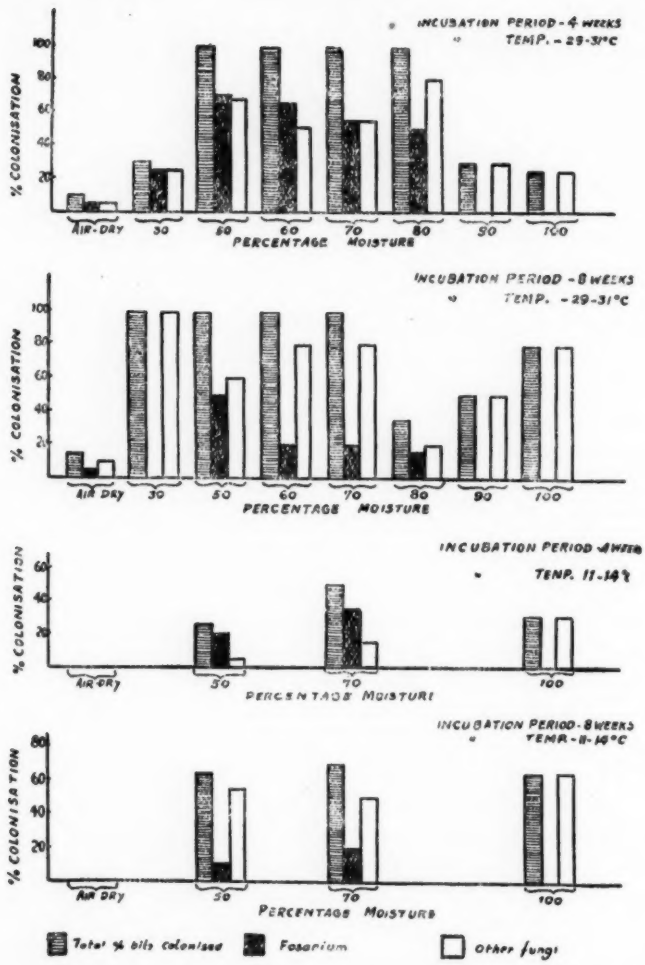
Experiment XII

This experiment, designed to study the effect of moisture and temperature on colonisation, was done using glass tumblers as containers for soils. One set incubated at room temperature (29-31° C.) was maintained at moisture levels of 30, 50, 60, 70, 80, 90 and 100 per cent. Another set, incubated at 11-14° C. in the refrigerator, was maintained at moisture levels of 50, 70 and 100 per cent. Air-dry soil served as control in both series. In the case of the controls with the air-dry soil, the mercuric chloride surface-sterilised root bits, after being washed in several changes of sterilised distilled water, were all dried between sterile blotting papers and then buried in the soil. The results are presented in Text-Fig. 2.

Conclusions.—(i) There was greater percentage *Fusarium* colonisation at 29-31° C. than at 11-14° C.

(ii) The effect of moisture on *Fusarium* colonisation was as follows:—In all cases, there was an increase in percentage colonisation up to the optimum moisture level, after which there was a fall in percentage colonisation. However, soil moisture percentage optimum for colonisation of root bits varied with other conditions. At 29-31° C. the optimum was 50 per cent. and there was no colonisation at 90 and 100 per cent. moisture levels. In the air-dry soil 5 per cent. colonisation was obtained. This is not surprising when it is realised that fresh cotton root bits by themselves contained some moisture which would permit at least an initial invasion of these root bits by the microflora. At 11-14° C. however, there was no colonisation in the air-dry soil and the percentage colonisation increased with the addition of moisture, maximum colonisation being obtained at 70 per cent. moisture level and there was again no colonisation at the 100 per cent. level. In the 30 per cent. moisture treatment at 29-31° C. 25 per cent. *Fusarium* colonisation was obtained at the end of 4 weeks, while at the end of 8 weeks, no *Fusarium* could be obtained. The saprophytic activity of the general microflora, on the other hand, reached a peak under the same conditions (Text-Fig. 2).

(iii) High soil moisture, *viz*., 90 and 100 per cent. evidently permitted and encouraged saprophytic activity of a microflora engaged in the root



TEXT-FIG. 2. Effect of soil moisture, temperature and period of incubation on colonisation by *Fusarium* and other fungi on cotton root bits buried and incubated in the Udamalpet "wilt-sick" soil.

decomposition to the exclusion of *Fusarium* spp. the saprophytic activity of this microflora reaching a maximum at the end of 8 weeks.

High soil moisture and low temperature, while they effected a certain curtailment in the saprophytic activity of *Fusarium* spp. as evinced by their colonisation on root bits, at the same time encouraged the development of

different secondary colonisers on the root bits. Thus, while high soil temperature and low soil moisture favoured secondary colonisation by *Aspergillus niger*, *Chætomium* sp. was found to be the dominant secondary, or even primary, coloniser at low temperature and high soil moisture, both conditions being unfavourable for general microbiological activity.

(iv) The rate of colonisation of the root bits generally increased with the period of incubation of root bits in soil. The rate of colonisation by individual members of the microflora, *e.g.*, *Fusarium* spp. did not, however, follow the same trend. Maximum colonisation by *Fusarium* spp. was obtained in all cases at the fourth week sampling, the eighth week sampling giving lower percentages of *Fusarium* colonisation (see Text-Fig. 2). This is indicative of the limited saprophytic activity of *Fusarium* spp. under the conditions of the experiment.

Experiment XIII

In order to study the relation of crop to *Fusarium* colonisation, four "wilt-sick" plots at Udamalpet (South India) were chosen. These were originally under KI (*Karunganni*) cotton from October 1944 to April 1945. In the following season the four different plots were under the following crops:

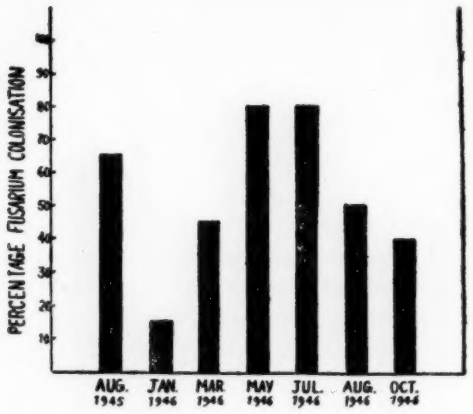
Plot No. 1: Cotton (KI);
„ 2: *Setaria italica* (Italian millet or *thenai*);
„ 3: *Cicer arietinum* (bengal gram);
„ 4: *Allium cepa* (onion) and *A. sativum* (garlic).

With a view to study the relation of season to colonisation, soil samples were obtained from each of the above plots at eight weekly intervals. Before burial, the root bits were sterilised at 20 pounds pressure for 30 minutes in 2 per cent. sodium nitrate solution, with a view to hasten *Fusarium* colonisation (Sadasivan, 1939). The limited space constrains the publication of all the data collected, hence only part of the results is presented in Text-Figs. 3 and 4.

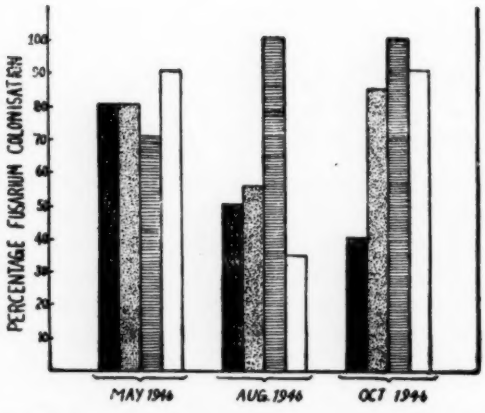
It will be seen from the results that differences in percentage *Fusarium* colonisation were obtained in the case of (i) soil collected from the same plot at different periods of the year (see Text-Fig. 3) and (ii) soil collected from the four different plots at the same period of the year (see Text-Fig. 4).

Experiment XIV

Fusarium colonisation in soils collected from different depths was next studied. Soil collected at various depths (down to 36 inches) from three



TEXT-FIG. 3. *Fusarium* colonisation on cotton root bits buried in "wilt-sick" soils collected from the cotton plot at different periods of the year.



TEXT-FIG. 4. *Fusarium* colonisation on cotton root bits buried in "wilt-sick" soils collected from three different plots at different periods of the year.

different plots, *viz.*, cotton, *Setaria* and onion was used. The results are incorporated in Table IX.

The results demonstrate the existence of *Fusarium* spp. down to a depth of 36 inches in " wilt-sick " soil. The percentage colonisation of *Fusarium* spp. and other fungi was found to vary according to the depth from which

TABLE IX

Percentage colonisation of Fusarium *spp. and 'other fungi' on cotton root bits buried in soils of various depths collected from three different wilt-infected plots at Udamalpet*

Soil depth in inches	Plot No. 1 (cotton)		Plot No. 2 (*Setaria*)		Plot No. 3 (onion)		Average of the three plots	
	Fusarium	Other fungi	*Fusarium*	Other fungi	*Fusarium*	Other fungi	*Fusarium*	Other fungi
0–6	90	55	100	100	90	90	93·30	81·7
6–12	100	100	100	85	90	100	96·67	95·0
12–18	100	45	100	80	75	50	91·70	58·3
18–24	85	100	95	100	85	..	88·30	100·0
24–30	100	100	90	100	60	50	83·30	83·3
30–36	75	15	75	50	75	40	75·00	38·3
Average ..	91·67	69·17	93·33	85·83	79·17	66·00	88·60	76·1

soil was collected. While the differences obtained in the average percentage *Fusarium* colonisation (see Table IX) in the case of soil from different plots may be due to the effect of particular crops on the fate of *Fusarium* in the soil, it is suggested here that the importance of the results just presented is to be sought in the continued persistence of *Fusarium* under different crops in the "wilt-sick" soil.

Experiment XV

Consideration of the high susceptibility of *Fusarium* to microbial antagonism suggested an investigation into the relation of the microflora already present in root bits and *Fusarium* colonisation. The importance of microbial antagonism in the colonisation of exposed wood surfaces has been well brought out by the investigations of Napper (1934) who found that *Fomes lignosus* cannot infect injured roots already colonised by a wound parasite such as *Ustulina zonata*, or other saprophytic fungi. It was, therefore, thought that *Fusarium* may not be able to effectively colonise a substratum already colonised by other organisms; cotton root bits were, therefore, given certain initial treatments, intended to encourage their colonisation by certain micro-organisms. Such treated root bits were buried in sterilised soil in litre flasks artificially infected with *Fusarium vasinfectum*. Fresh cotton root bits soaked in a 3 per cent. solution of sodium nitrate (intended to

supply sufficient nitrogen so as to facilitate invasion of the root bits by a microflora), were sterilised by autoclaving at 20 pounds pressure for 30 minutes. One set of such autoclaved root bits was kept free from outside contaminations; another set was subjected to contamination and colonisation by the wild aerial microflora. The root bits used in treatments 3 and 4 were old and were subjected to preliminary colonisation and decomposition by the aerial microflora for some period after collection. Fresh root bits were used for treatments 5 and 6, and these served as controls. The root bits were buried and incubated in *F. vasinfectum* inoculated soil, after which they were removed and plated out as usual. Table X gives the results.

TABLE X

Percentage colonisation of Fusarium *and other fungi on variously treated cotton root bits buried in* Fusarium *soil*

Treatment	% Root bits yielding *Fusarium*	% Root bits yielding other fungi	List of other fungi
1. Sterilised root bits with added nitrogen : colonised by aerial wild fungus flora and buried as such	85	100	*Aspergillus niger*., *Rhizopus* sp.
2. Sterilised root bits with added nitrogen : not colonised by wild fungus flora and buried in sterile condition	100	Nil	Nil
3. Unsterilised old root bits: not surface sterilised before burial	95	100	*Aspergillus niger*, unidentified fungus
4. Unsterilised old root bits : surface sterilised before burial	100	100	*Aspergillus niger*,
5. Fresh root bits : not surface sterilised before burial	100	75	*Aspergillus niger*,
6. Fresh root bits: surface sterilised before burial	100	Nil	Nil

The following conclusions may be drawn:

(i) *Fusarium vasinfectum* colonised the root bits in all the treatments. While 100 per cent. colonisation was obtained in the case of treatments 2, 4, 5 and 6, in the two other treatments the percentage colonisation was slightly less.

(ii) The root bits used in treatments 2 and 6 were free from all microflora, hence the 100 per cent. colonisation of *Fusarium* obtained in the case of these treatments.

(iii) That the flora on the root bits might have an influence, however small, on *Fusarium* colonisation is also evident from the percentage *Fusarium* colonisation obtained in the case of treatments 1 and 3, where the root bits

were not surface sterilised before burial and resulted in slightly lower percentage of *Fusarium* colonisation. The internal microflora of the surface sterilised root bits in treatment 4, however, was not sufficient to affect *Fusarium* colonisation.

Although the lower percentage of *Fusarium* colonisation obtained in treatments 1 and 3 may be attributed to the effect of the microflora of these root bits, it is quite possible that the lower percentage *Fusarium* colonisation obtained was a result of change in composition of the root bits due to partial decomposition of the same by the microflora.

Experiment XVI

In this experiment a study was made of the relative colonising capacity of *Fusarium vasinfectum* in the presence of certain other soil fungi. The experimental method consisted in growing *Fusarium vasinfectum* and other soil fungi in sterilised compost soil separately, mixing one of the soil fungus cultures with a *Fusarium* culture and burying root bits therein. The root bits were finally plated out as usual. The soil fungi used were: (*a*) *Aspergillus niger*, a fungus common to Udamalpet soil and invariably found as a dominant secondary coloniser of cotton root bits; (*b*) *Rhizoctonia bataticola*, an isolate of the fungus obtained from diseased cotton plants and noted for its scant colonising capacity; (*c*) *Dactylaria* sp.; (*d*) *Trichoderma lignorum*; and (*e*) mixed flora (inoculation with a mixed flora was done by transferring to sterilised soil one gram unsterilised compost soil). The results are presented in Table XI.

TABLE XI

Relative colonising capacity of Fusarium vasinfectum *and other soil fungi*

No.	Combination of fungi	% *Fusarium* colonisation	% Colonisation of the other fungus or fungi
1	*Fusarium* alone ..	100	Nil
2	,, + *Rhizoctonia bataticola* ..	100	Nil
3	,, + *Trichoderma lignorum* ..	Nil	100
4	,, + *Dactylaria* sp. ..	Nil	100
5	,, + *Aspergillus niger* ..	Nil	100
6	,, + Mixed flora ..	10	100*

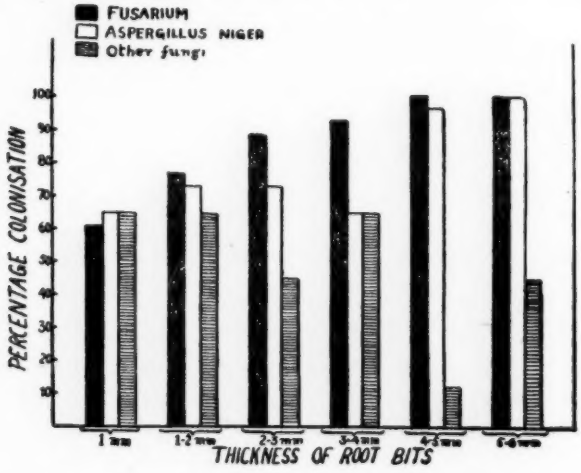
* The fungal colonisers were: *Aspergillus niger*, 100%; *Cunninghamella echinulata*, 20%; *Rhizopus* sp., 30%.

The results show that while *Fusarium* invariably colonised root bits buried in natural unsterilised soil with its component of a complex microflora, in sterilised soils inoculated with individual species it was not always

able to do so. In the presence of *Dactylaria* sp., and *Trichoderma lignorum* for instance, *Fusarium* could not colonise the root bits. Of the other individual species included in the present study, *Rhizoctonia bataticola* is noted for its scant colonising capacity and as such it is but natural that it was not able to colonise root bits in the presence of *Fusarium*. On the other hand, *Aspergillus niger* which, as we have seen already, proved a dominant secondary coloniser of root bits, was found to be able to colonise 100 per cent. of the root bits in the presence of *Fusarium* to the complete exclusion of *Fusarium*. However, in the presence of a mixed flora containing inoculum of *Aspergillus niger*, *Fusarium* continued to colonise root bits.

Experiment XVII

The effect of root size on *Fusarium* colonisation was next taken up for study. Bits were cut out of roots of cotton plants and graded, according to size, into six classes, *viz.*, 1 mm., 1-2 mm., 2-3 mm., 3-4 mm., 4-5 mm., and 5-6 mm. thick. The graded root bits were buried separately in soil. The results are given in Text-Fig. 5. It will be seen that *Fusarium* spp. colonise the root bits irrespective of their size.



TEXT-FIG. 5. Colonisation by *Fusarium* and other soil fungi on cotton root bits of different sizes.

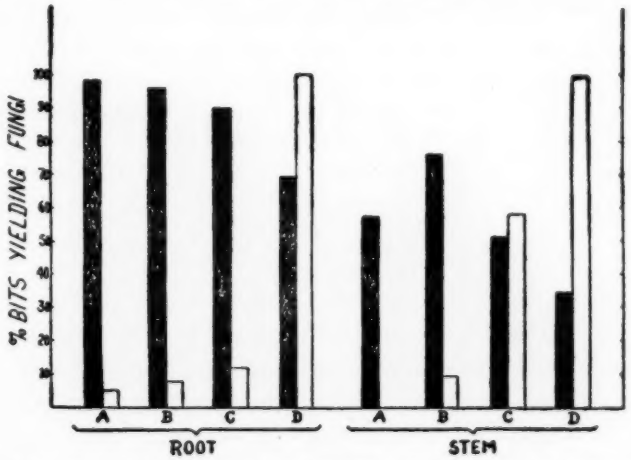
Succession of Fungi Decomposing Cotton Root Bits in Udamalpet Soil with Special Reference to Fusarium *spp.*

Wilted cotton plants from Udamalpet (S. India) gave out in culture one or more fungi other than the pathogenic *Fusarium*. These fungi were

vidently secondary invaders, resembling the succession of fungi developed in the underground parts of plants after infection by a primary parasite reported by a number of workers (Samuel and Garrett, 1932; Hildebrand, 1934; Koch, 1935; Hildebrand and Koch, 1936; Sadasivan, 1939). In one of the experiments an attempt was made to study the fungi that were associated with wilted cotton plants with special reference to " secondary invaders", if any. Wilted cotton (*Karunganni*) plants collected from Udamalpe were divided into four lots as follows:—

1.	Plants just wilting ..	designated as	'A' group
2.	Plants just wilted ..	"	'B' "
3.	Plants wilted and all leaves shed ..	"	'C' "
4.	Plants wilted and aerial parts dried up ..	"	'D' "

This classification was intended to give results which would indicate the nature of the micro-organisms associated with cotton plants in various stages of wilt and decomposition, the expectation being that the 'A' group of plants would be comparatively free from secondary organisms which would gradually become dominant as we proceed to group 'D'. Root and stem bits from the four groups of plants were plated out in separate lots on three different media (potato-dextrose, Horne and Mitter's, and Barnes' agars). The results which are presented in Text-Fig. 6 showed the development of



TEXT-FIG. 6. Percentage root and stem bits from the 'A', 'B', 'C' and 'D' groups o wilted plants yielding *Fusarium* and other fungi.

a succession of fungi on infected and wilted parts of cotton plants. Plants in group 'A' were practically free from invasion by any fungi other than *Fusarium*, which is the primary parasite in the present case. The 'C' and 'D' group of plants, on the other hand, yielded some fungi other than the parasite and these should, therefore, be regarded as secondary invaders. The primary parasite, *viz.*, *Fusarium vasinfectum* showed some tendency to disappear gradually from wilted plants, with the progressive invasion of the same by secondary fungi (Text-Fig. 6). It is, therefore, clear that a succession of fungi appears on underground parts of cotton plants, after the living plants are attacked and wilted by the primary parasite. The following fungi, in order of their appearance, were dominant in the above fungal succession:—*Aspergillus niger*; *A. nidulans* Group; *A. terreus* Group; *Rhizopus* sp. ; *Aspergillus fumigatus* Group.

Apart from the results just enumerated, preliminary work on the colonisation of healthy cotton root bits buried in " wilt-sick " soil (Subramanian, 1946 *b*) had also indicated the occurrence of a fungal succession on plant roots buried in soils. The study of this aspect of the problem was next taken up using the root burial technique.

Experiment XVIII

In this experiment a study was made of the fungal succession on cotton root bits buried in the Udamalpet " wilt-sick " soil collected from the cotton

TABLE XII

Colonisation by Fusarium *spp. and by secondary colonisers on cotton root bits in Udamalpet " wilt-sick " soils collected at different seasons*

Season of soil collection	*Fusarium* spp.						Secondary colonisers					
	Time in weeks						Time in weeks					
	1	3	6	8	12	16	1	3	6	8	12	16
January ..	65	85	100	..	100	100	75	70	90	..	100	100
March ..	20	55	85	..	100	100	60	65	100	..	100	100
May ..	35	45	85	100	100	100	0	0	35	70	100	100
July ..	30	60	100	100	100	100	0	5	55	100	100	100
August ..	30	60	85	95	100	100	35	100	100	100	100	100
October ..	15	20	45	65	..	100	25	70	100	100	..	100

plot (plot no. 1) at eight-weekly intervals during the year. The plot was sown to cotton throughout the sampling period except during the fallow months. The results presented in Table XII includes only some of the weekly observations made on the colonisation of the fungi on root bits since not much variation was seen at shorter periods of incubation.

The results indicate the following conclusions:

(i) *Fusarium* spp. were primary and dominant colonisers on root bits and showed progressive increase in percentage colonisation. After attaining maximum colonisation it remained at peak level during the rest of the period of study, *i.e.*, 16 weeks.

(ii) More important among the secondary fungal colonisers on root bits were three species of *Aspergillus*, viz., *A. niger*, *A. nidulans* Group (1), *A. fumigatus* Group. Thus, the nature of the secondary microflora developing on the buried root bits resembled the natural succession of fungi found to occur on wilted cotton plants in various stages of disease and decay (see Expt. XVII).

(iii) Among the chief fungal colonisers, the sequence of fungal succession, in order of their appearance, was as follows: *Fusarium* spp.—*Aspergillus niger*—*A. nidulans* Group—*A. fumigatus* Group.

(iv) While the progress of *Fusarium* colonisation was steady, that of the secondary colonisers was erratic. The data did not indicate any definite correlation between percentage *Fusarium* colonisation and the activity of the secondary colonisers.

(v) The above results have also shown varying percentages of *Fusarium* colonisation in different seasons.

Experiment XIX

The effect of certain soil amendments on *Fusarium* colonisation and the fungal succession on cotton roots was then studied. The following materials, at the rate of 2 per cent. on weight basis, were used to amend Udamalpet soil collected in August 1945:—(i) ammonium sulphate; (ii) sodium nitrate; (iii) stable manure; (iv) starch (soluble); (v) potassium acid phosphate; (vi) calcium phophate (superphosphate); (vii) potassium sulphate; (viii) potassium carbonate; and (ix) lime (calcium hydroxide). Unamended soil served as control. The fungal succession on the root bits was studied for a period of six weeks. The results are summarised in Table XIII.

TABLE XIII

Effect of certain soil amendments on the percentage colonisation of Fusarium *spp.*, Aspergillus niger *and 'other fungi' on cotton root bits in Udamalpet "wilt-sick" soil*

Soil Amendments	Details of colonisation	Sampling period in weeks					
		1	2	3	4	5	6
None : Control	*Fusarium*	35	40	45	52	65	65
	A. niger	100	100	100	100	100	100
	'Other fungi'	55	29	25	0	15	37
$(NH_4)_2SO_4$	*Fusarium*	70	100	76	46	37	76
	A. niger	100	95	100	100	100	87
	'Other fungi'	70	53	50	48	43	44
$NaNO_3$	*Fusarium*	20	80	94	100	86	85
	A. niger	100	100	95	100	100	100
	'Other fungi'	36	94	78	54	33	35
Stable manure	*Fusarium*	26	34	40	21	27	29
	A. niger	100	90	89	100	100	100
	'Other fungi'	83	60	0	0	11	49
Starch	*Fusarium*	39	52	40	50	85	75
	A. niger	100	100	85	100	76	100
	'Other fungi'	52	23	47	34	56	50
KH_2PO_4	*Fusarium*	65	62	90	42	21	91
	A. niger	100	100	100	100	100	100
	'Other fungi'	66	49	75	4	0	11
Super-phosphate	*Fusarium*	60	42	33	36	47	65
	A. niger	100	100	100	100	100	100
	'Other fungi'	53	5	25	12	25	29
K_2SO_4	*Fusarium*	85	35	74	35	80	83
	A. niger	90	100	100	100	100	100
	'Other fungi'	41	12	26	15	31	18
K_2CO_3	*Fusarium*	55	84	63	66	50	89
	A. niger	95	100	83	100	100	89
	'Other fungi'	82	9	49	15	79	100
$Ca(OH)_2$	*Fusarium*	90	100	70	55	94	97
	A. niger	65	100	84	76	94	56
	'Other fungi'	100	85	30	100	54	100

All the amendments used affected the percentage colonisation by *Fusarium* spp. and also the fungal succession on root bits.

Inorganic nitrogen in both forms, *i.e.*, ammonium sulphate and sodium nitrate tended to enhance the colonisation of the root bits by *Fusarium* and also by secondary fungi (see Table XIII). The rate of

colonisation of *Fusarium* spp. in the case of ammonium sulphate was more rapid than in the case of sodium nitrate; the rate of *Fusarium* colonisation was, however, more steady in the case of the latter. In the case of both these nitrogen sources therefore, the activity of *Fusarium* spp. during the initial stages of colonisation was never held in check but was heightened.

Stable manure had a depressing effect on colonisation (see Table XIII). Starch, on the other hand, enhanced both the degree and rate of colonisation of *Fusarium* spp. on root bits although not to such an extent as inorganic nitrogen. The activity of the general microflora as indicated by the colonisation percentage of other fungi was also greater in the case of starch as compared with the control.

Phosphate increased *Fusarium* colonisation on root bits. Potassium acid phosphate gave higher figures for *Fusarium* colonisation and also for secondary colonisers, than superphosphate.

The application of potassium to the soil in the form of both sulphate and carbonate contributed towards better *Fusarium* colonisation than was obtained in the control, although the progress in colonisation was not steady. The activity of secondary colonisers was greater in the case of potassium supplied as carbonate.

The saprophytic activity of *Fusarium* spp. and other fungi was enhanced to a very great extent by the addition of 2 per cent. calcium hydroxide to the soil. Indeed, the saprophytic activity of *Fusarium* spp. as evidenced by its capacity to colonise root bits was at its highest in this case.

Among the secondary fungal colonisers, *Aspergillus niger* was the dominant form. The more important secondary colonisers from the point of view of percentage occurrence in all the treatments put together were:

Aspergillus flavus Group	(Percentage	occurrence	63·3)
A. terreus Group	(„	„	41·67)
A. nidulans Group (1)	(„	„	38·33)
A. nidulans Group (2)	(„	„	30·00)
Curvularia sp.	(„	„	21·67)
Rhizopus sp.	(„	„	16·67)
Chætomium sp.	(„	„	16·67)

Discussion

The present work which is a continuation of studies already reported (Subramanian, 1946*a*, 1946 *b*) has confirmed the writer's previous observations on the growth, survival and perennation of *Fusarium vasinfectum* in its soil environment. Although a number of past workers (Fulton, 1907; Orton, 1908; Hibbard, 1910; Butler, 1933) suggested free spread of *F. vasinfectum* through soil, the experimental data presented here do not support these observations. On the other hand, the results obtained in this investigation showed that *F. vasinfectum* is incapable of making free growth in unsterilised soils under ordinary conditions (Expt. VII). *F. vasinfectum* thus belongs to Garrett's (1944) category of fungi which make no extensive growth in soils. It would appear that subterranean spread of this fungus is confined to growth internal to the host and that disease spread is brought about by root contact.

The inability of *F. vasinfectum* to grow freely in natural soils could be attributed to two causes: (i) the antagonism of the soil microflora to the fungus; and (ii) the accumulation of soil carbon dioxide and its inhibiting effect on fungal growth in soil (Garrett, 1936; Blair, 1943). The author's own previous work pointed to the former as the possible cause, since the fungus was found to grow and sporulate freely in sterilised soil. Nevertheless, clarification was necessary regarding carbon dioxide as a possible factor retarding fungal growth in soils. The problem was, therefore, investigated in detail. Study in pure culture of two strains of *Fusarium vasinfectum* and of other soil fungi which differed from them in their ability to make free growth in soils, *viz.*, *Pythium aphanidermatum* (Sadasivan, 1946 unpublished), *Rhizoctonia bataticola* (Kovoor, 1947, unpublished) and *Dactylaria* sp. (Subramanian, 1947, unpublished) showed that growth of *F. vasinfectum* and *Pythium aphanidermatum* was inhibited considerably by carbon dioxide, while that of the others was not (Table V). Thus, in the case of the fungi studied there was no correlation between ability or inability to make free growth in natural soils and their inhibition by carbon dioxide in pure culture. Moreover, the removal of carbon dioxide from unsterilised soil did not facilitate the growth of *F. vasinfectum* therein (Expt. VI). These findings, coupled with the fact that carbon dioxide concentrations in the soil "microclimate" seldom reaches even one per cent. under normal conditions (Russell & Appleyard, 1915), suggest that carbon dioxide inhibition may not be a factor delimiting fungal growth in bare soil. On the other hand, the present investigation indicates microbial antagonism

(Plates IV and V, Table 6) as the factor mainly responsible for delimiting growth and survival of *F. vasinfectum* in unsterilised soil. Thus, survival of fungi in soils is closely linked up with their ability to make free growth in soils since "vegetative fungus mycelium does not persist in the soil unchanged; if it is not actively growing, it must be decomposing" (Garrett, 1936, p. 669).

These observations relating to the limited survival of *Fusarium vasinfectum* in natural soils indicate the possibility of cotton wilt control by elimination of the pathogen from the soil. Further investigation into this question showed that the exit of the fungus from soil was hastened by factors which increased the general microbiological activity of the soil. Disappearance of *F. vasinfectum* from the soil was delayed by absence of moisture in air-dry soil (Table I), by low temperature (Table I) and to a certain extent by anaerobic conditions produced in the laboratory by adding water to soil upto 100% moisture-holding capacity (Table I). On the other hand, the disappearance of the fungus was hastened by forced aeration (Text-Fig. 1), by high temperature (Table I) and by addition of stable manure (Expt. VI; see also Subramanian, 1946 *a*). The rate of disappearance of *Fusarium vasinfectum* was also affected by the nature of the soil flora (Table II). Complete exclusion of micro-organisms by sterilisation of soil resulted in indefinite survival of the fungus (Expt. X). It, therefore, appears that the rate of disappearance of *F. vasinfectum* from the soil depends mainly on the nature, numbers and activity of the antagonistic micro-organisms in the soil, its actual survival in "wilt-sick" soils being accomplished by colonisation on cotton root bits and other stubble left in the field. Experiments using "wilt-sick" soils showed that cotton root bits were colonised by *Fusarium* when buried in these soils and the fungus continued as a primary and dominant coloniser for over 16 weeks under ordinary conditions (Table XII). The fungus had no specificity in the matter of colonisation on plant substrata and colonised roots, stems and petioles of other plants (Table VIII). It was also noticed that stubble collected from "wilt-sick" fields yielded in culture large number of *Fusaria*. A detailed investigation into the factors affecting *Fusarium* colonisation on cotton root bits showed that *Fusarium* colonisation was retarded to some extent by (i) low temperature (Text-Fig. 2), (ii) lack of moisture, high moisture, *viz.*, 90 and 100 per cent. (Text-Fig. 2) and by addition of stable manure (Table XIII). *Fusarium* could colonise root bits irrespective of their size (Text-Fig. 5). It could also colonise bits already colonised by other fungi (Table X). *Fusarium* was also found to be able to colonise root bits when buried in "wilt-sick" soils collected (1) at different seasons (Table XII, Text-Fig. 3), (2) from different depths (Table IX) and (3) from

plots under different crops (Text-Fig. 4). Soil amendments, while having some effect on the extent and progress of *Fusarium* colonisation, did not prevent *Fusarium* colonisation altogether (Table XIII). These findings emphasise the ability and tendency of *Fusarium* to colonise on stubble and persist under widely different conditions, thus ensuring its continued survival in soils for long periods. On the basis of these data *F. vasinfectum* is considered a soil inhabitant resembling *F. culmorum* (Sadasivan, 1939). It would appear that *F. vasinfectum* possesses the ability to colonise and to utilise plant residues to a greater extent than other soil saprophytic organisms with which it is generally thrown into competition. This ready preference by this fungus of a substratum during its period of dormancy may be the expression of a device to reduce its vulnerability to microbial antagonism and remain inside the stubble in a state of passive saprophytism simulating what is generally obtainable under pure culture conditions.

It should be mentioned, in conclusion, that the observations made in this paper regarding the behaviour of *Fusarium vasinfectum* in soils has an important bearing on the field control of cotton wilt in this country. The possibility of biological control of soil-borne wilt pathogens is not a new idea; the practical application of this concept has been demonstrated in the control of *Phymatotrichum* root rot of cotton in the United States (King, Hope and Eaton, 1934; Eaton and King, 1934; King, 1937). In this connection, the susceptibility of *Fusarium vasinfectum* to microbial antagonism coupled with the demonstration of its rapid disappearance from soils especially under conditions of forced aeration (Text-Fig. 1), and soil amendment with stable manure in the presence of moisture (Subramanian, 1946 *a*) indicate practical possibilities. Modification of physical or chemical conditions of the soils so as to raise the general microbiological activity to a high level would result in the gradual elimination of the pathogen from " wilt-sick " soils. Unfortunately, the saprophytic survival of *F. vasinfectum* as a coloniser on stubble complicates the whole problem of wilt control since the duration of its persistence in soils of low or high microbiological activity cannot be predicted with any degree of accuracy. The present investigation only points to the complexity of the problem and doubtless future investigations should be aimed at the elimination of *F. vasinfectum* from its organic substratum.

Summary

1. *Fusarium vasinfectum* did not make any growth in unsterilised soils, due to the antagonism of the microflora; the fungus, on the other hand, was attacked and decomposed by antagonistic organisms, especially bacteria.

2. Under ordinary conditions the persistence of *F. vasinfectum* in soil was limited by the operation of the microbial factor. The fungus was more tolerant of adverse physical conditions of the soil than those favourable for general microbiological activity. Its disappearance from soil was particularly hastened under a wide range of moisture conditions (30–80 per cent.), more especially at 30 per cent., by fairly high temperature (28–30° C.), by forced aeration, and by addition of stable manure. Persistence of the fungus in the soil was favoured by lack of moisture and excessive moisture, and continued undiminished under sterile conditions for a period exceeding twenty-four months.

3. The experimental results suggest that the inability of *Fusarium vasinfectum* to make growth in unsterilised soil is not so much due to its inhibition by soil carbon dioxide but due to microbial antagonism.

4. *Fusarium* was a primary and dominant coloniser of various plant parts buried in moist " wilt-sick " soils from Udamalpet (S. India); invariably it continued as a dominant coloniser for a period of 16 weeks on the root bits. The unlimited saprophytic potentialities of *F. vasinfectum* are considered sufficient to justify its inclusion into the class of true *soil fungi* or *soil inhabitants*.

5. *Fusarium* colonisation on cotton roots was partially or completely inhibited by absence of moisture, excessive moisture resulting in water-logging, by low temperature and by organic manuring. Under all other conditions colonisation took place to a greater or smaller extent. It was favoured by addition of lime to soil. *Fusarium* was, moreover, able to colonise cotton root bits irrespective of their size or the presence of any microflora already present in the root bits.

The results of a study of the relative colonising capacity of *Fusarium vasinfectum* and other soil fungi are presented.

6. *Fusarium* colonisation in soils under different crops was not appreciably affected by the nature of the crop; in all cases the pathogen continued to persist in soils irrespective of the nature of the crop.

No correlation could be obtained between the relative colonising capacity of *Fusarium* and other fungi in soils collected at different seasons.

7. Experiments using " wilt-sick " soil from three different plots (cotton, *Setaria* and onion), collected at different depths, showed that *Fusarium* had a deep vertical distribution in soil, extending down to thirty-six inches.

8. Details are given of the succession of fungi decomposing cotton roots buried in Udamalpet "wilt-sick" soil (*a*) collected at different periods of the year, and (*b*) in the presence of certain organic and inorganic amendments.

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Explanation of Plates

PLATE IV

(Photomicrographs showing antagonism of micro-organisms to *Fusarium vasinfectum*)

Fig. 1. Healthy hypha of *Fusarium vasinfectum* being attacked by bacteria. Note that the hypha is entire but appears corroded in the region indicated by the arrow, due to the action of the bacteria (× 530).

Fig. 2. Early stage in decomposition of *F. vasinfectum* hypha by bacteria. Arrow points to a portion of the hypha which has undergone complete decomposition by the bacteria (× 550).

Fig. 3. Hyphae of *F. vasinfectum* completely enveloped by bacteria. It is noteworthy that the bacteria are not distributed all over, but are found closely crowding around *Fusarium* hyphae (× 350).

Fig. 4. Final stage in the decomposition of *F. vasinfectum* hyphae by bacteria. Note again the localized aggregation of bacteria around hyphae or, when these are absent, around linear vacant spaces previously occupied by these hyphae (× 270).

Fig. 5. A still advanced stage in the decomposition of *F. vasinfectum* hyphae. Note the scattered (not localized) distribution of bacteria. The faint hyphal outlines indicate the position previously occupied by the healthy (now completely decomposed) hyphae. (× 350).

Fig. 6. Decomposition of macroconidia of *F. vasinfectum* by bacteria : a conidial cluster which has undergone complete decomposition (× 890).

PLATE V

Fig. 7. Shows a microconidial cluster which has undergone complete decomposition by bacteria. The vacant spaces correspond in outline to the microconidia which have been decomposed. Note that two or three conidia are still left undecomposed and hence well stained (× 510).

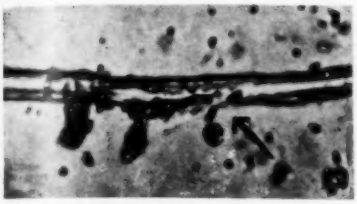
Fig. 8. Undecomposed microconidia of *F. vasinfectum* among decomposing hyphae. The conidia are darkly stained and healthy while the hyphae are poorly stained and faint in outline (× 350).

Fig. 9. Decomposing hyphae of *F. vasinfectum* with intercalary chlamydospores which are undecomposed and hence well stained (× 550).

Fig. 10. Decomposition of chlamydospores of *F. vasinfectum* by bacteria (× 780).

Fig. 11. Decomposition of chlamydospores by antagonistic filamentous soil micro-organisms (actinomycetes ?). Note that the chlamydospore wall has undergone partial decomposition (× 800).

Fig. 12. Bacteria attacking the promycelia of germinating chlamydospores and conidia of *F. vasinfectum*. Arrows indicate points of attack on the promycelia (× 890).



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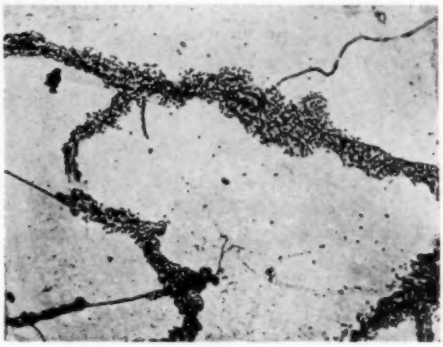

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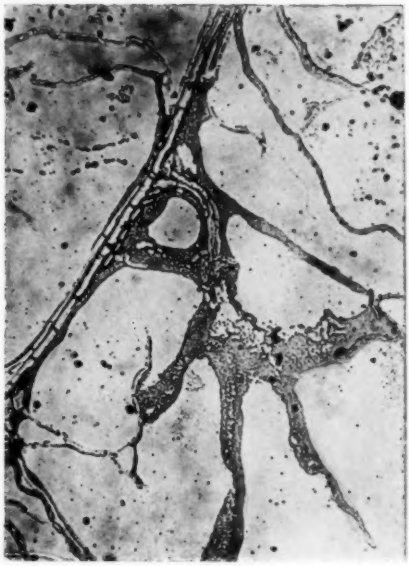

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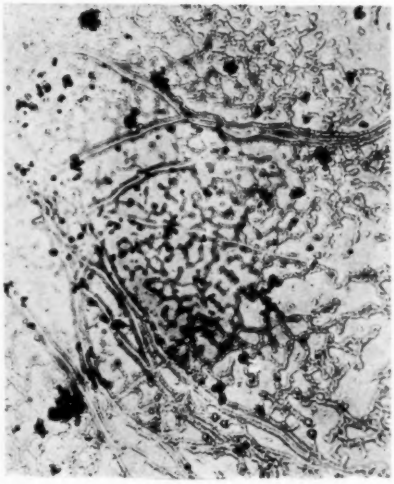

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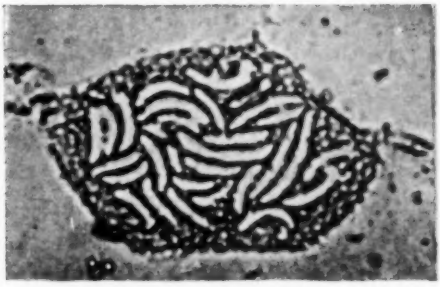
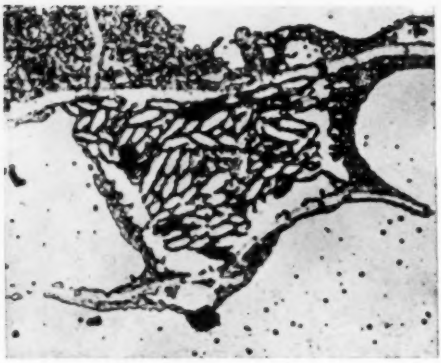


6

Soil Conditions and Wilt Diseases in Plants



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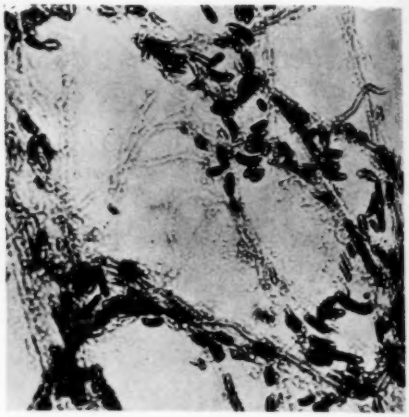

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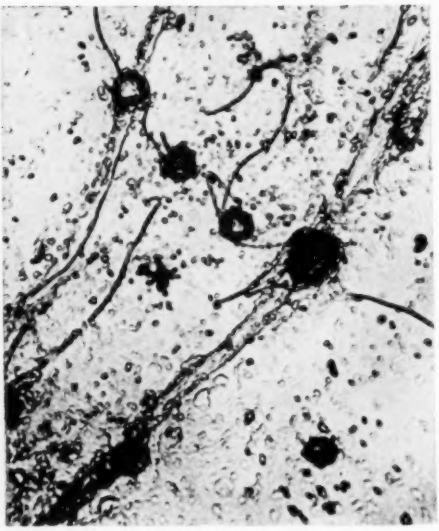

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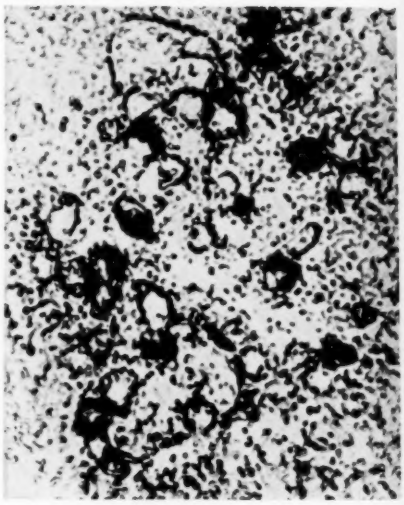

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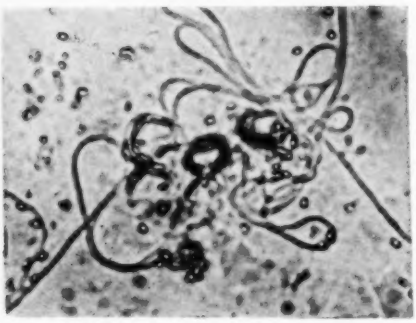

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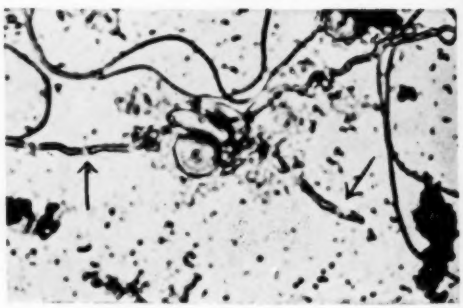

12

Soil Conditions and Wilt Diseases in Plants

ERGOT PRODUCTION AND IMPROVEMENT

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Received March 5, 1949

Ergot of rye of commerce is the sclerotium developed by the fungus *Claviceps purpurea* Tul., on the ears of rye replacing some of the grains. It is an important drug particularly useful in the treatment of *post partum* hæmorrhage. The world's supply of this drug was for a long time obtained from Spain, Portugal or Russia. Natural infection of the rye crop cultivated in these countries was mainly responsible for the availability of the drug in Europe. Rye is not cultivated to any extent in India. The requirements of this drug in India depended entirely on imports. During the World War II there was cessation of the supplies of ergot and it was not available even in several hospitals. At this juncture the Madras Agricultural Department initiated the artificial production of ergot on the Nilgiris (Thomas and Ramakrishnan, 1942) and made it available to Indian pharmaceutical firms. The factors influencing infection and quality of ergot on the Nilgiris were investigated and the results are recorded in this communication.

Materials and Methods

A limited area at the Agricultural Research Station, Nanjanad, was being cultivated with a variety of rye whose origin had been traced to Italy. This was the nucleus host material which was multiplied in later years. Other varieties were also obtained from the Agricultural Departments of New South Wales and Kashmir and from the Head of the Division of Botany, Agricultural Research Institute, New Delhi, for comparative trials. Fresh cultures of *Claviceps purpurea* were obtained through the courtesy of Dr. Magee and Mr. I. A. Watson of Australia. These were multiplied for later use. The analyses of sclerotia for determination of the alkaloid content were made according to the method described by Mukerji and De (1944). Inoculation of the rye crop was effected by spraying the spore-suspension on opening flowers with the help of stirrup pumps or Hyject sprayers. Five bottles of the culture were used to spray an acre once. The sclerotia were harvested by hand picking.

Experiments

Multiplication of culture of the fungus.—The stock culture of the fungus was maintained on Kirchoff's agar media (1929). Comparison of the

relative growths of the fungus on media with asparagin or peptone as the source of nitrogen showed that the latter substance can be used to replace asparagin without any disadvantage. For purposes of spray inoculation the cultures were grown on sterilised rye grains in ordinary pint bottles. The bottles were filled to one-third the capacity with clean rye grains and an equal quantity of water was added. They were plugged and autoclaved for 40 to 45 minutes to ensure complete sterilisation. This was found to be the best medium. Mixtures of equal quantities of wheat and rye or oats and rye also produced good growth of fungus.

Two methods of inoculation of the media in the bottles were tried. In one series bits of agar cultures 0·5 cm. in diameter were placed on the sterilised grains and the bottle was thoroughly shaken to mix the inoculum with the grains. In the second series a heavy suspension of the spores was made by mixing a similar bit of the culture with sterilised water in test-tubes. Two cubic centimetres of this suspension were poured into a bottle of sterilised grains and shaken well. The second method was found to be better. A satisfactory growth developed within a fortnight while in the former it took over a month to grow through the entire medium. Thus the latter method helped to save time.

Altitude and growth of rye.—Rye is a crop of the temperate zone. In order to test the most suitable elevation in this province for the cultivation of rye it was sown in 5 different places, *viz.*, Coimbatore (1,400 ft.), Knoll Estate (Nilgiris, 4,000 ft.), Coonoor (5,600 ft.), Keti (6,500 ft.), Nanjanad (7,000 ft.). In all places the sowings were made in June to July. The plants dried up before reaching the flowering stage at Coimbatore. At Knoll Estate the growth was poor and seed setting unsatisfactory. In Coonoor and higher elevations the growth was very good and the yield of grain was satisfactory. From these observations it was decided that the most suitable places for rye cultivation for ergot production in the province must be at an elevation of 5,600 ft. or over and later sowings were confined to the upper elevations of the Nilgiris and Kodaikanal.

Variety of rye and infection.—A comparison was made of the different varieties of rye available with us to find out the most satisfactory variety for maximum production of ergot. Twelve varieties were under trial. These were grown in replicated quarter-cent. plots as only small quantities of the seed could be obtained and uniformly sprayed with spore suspension. The ergots produced were harvested dried and weighed. The results are recorded in Table I.

TABLE I

Statement showing the nature of growth and yield of ergot of different varieties of rye

Variety	Nature of growth	Average yield of ergot in grams
Af. 31-2 ..	Slow growing few tillers	7
Af. 32-2 ..	do	12
Af. 32-3 ..	do	15
Af. 32-5 ..	do	13
Af. 33-1 ..	do	16
Af. 34-2 ..	do	18
Af. 36-3 ..	do	16
Af. 12-2 ..	do	6
Af. 306 ..	do	15
Kashmir ..	Bushy prostrate-few tillers slow growing	20
Black winter rye ..	Quick growth-erect, satisfactory tillering	98
Local rye ..	Erect, many tillers, taller than black winter rye	88

The Australian black winter rye and the local rye were found to be the most promising varieties for ergot production. Hence for large-scale production of ergot these two varieties only were used.

Time of sowing and infection.—It has been observed in other countries that humid weather favours ergot infection. With a view to find out the most favourable period for the cultivation of rye for ergot production rye was sown in one cent. plots every month beginning from January to December 1943. The plants were inoculated when flower opening commenced by spraying the spore suspension twice a week. Eight sprayings were given to each plot. The ergot was handpicked when mature and the dry weight determined. Ttable II gives the harvest figures.

TABLE II

Statement showing the date of sowing and yield of ergot

Date of sowing	Date of flower opening	Dry weight of ergot in grams	Remarks
6—1—1943	26—4—1943	85	
6—2—1943	20—5—1943	96	
6—3—1943	23—6—1943	140	
6—4—1943	2—7—1943	278	
6—5—1943	13—8—1943	231	
6—6—1943	31—8—1943	198	
6—7—1943	15-10—1943	143	
6—8—1943	17-11—1943	118	
6—9—1943	16-12—1943	98	
6-10—1943	15—2—1944	10	
6-11—1943	15—3—1944	15	Growth sparse few sclerotia
6-12—1943	7—4—1944	5	"

It was observed that good yields were obtained when rye was sown between April and June. But the maximum yields were from April sowings and generally cereals are sown on the hills in April soon after the receipt of summer showers. The results showed that for commercial production of ergot sowings after August were not advisable. An examination of the rainfall records during the flowering period showed a positive correlation between the number of rainy days in the month of flowering and the yield of ergot. In the months of July to October there were 20 or more rainy days in the month in 1943, and the plots which flowered during this period gave the best yield. Between December and April the maximum number of rainy days were only six per month and during the rest of the period the weather was bright and sunny with the result that infection was very low or negligible. Thus it becomes evident that under normal conditions of rainfall it is advantageous to sow in April. Experience gained in later years have brought the close relationship between the number of rainy days during the flowering period and intensity of infection into more prominence. Spells of misty weather with light drizzle have proved very beneficial for spread of infection. But heavy rain during the maturing stages of ergot have resulted in causing lodging of rye and shedding of ergot.

Frequency of spray inoculations and intensity of infection.—The rye field was divided into one cent. plots. The number of spray inoculations was varied from two to eight in different plots. But in all cases the inoculations were commenced only after anthesis. The percentage of infected tillers were recorded in the different plots and the average calculated. It was observed that higher incidence of infection was evident with increase in the frequency of sprays. There was no difference between the plots receiving six sprays and those sprayed eight times. The average percentages of infection are recorded in Table III.

TABLE III

Frequency of sprays and intensity of infection

Number of spray inoculations	Percentage of infection
2	58
4	65
6	70
8	71

When to spray.—The spray inoculations were conducted at different periods after the emergence of the ear in order to determine the proper time for spraying. The plants were raised in isolated plots and the inoculations were made in the first, second, third, fourth and fifth weeks of the emergence of ears outside the shot blade. The infection was very low in the plots which were sprayed in the first or fifth week of emergence. The highest infection occurred in plots sprayed in the third week. It must be mentioned that during this period flower opening was at its peak with the anthers dangling out from most of the spikelets. The presence of open flowers was found to be the most significant factor. In the first week the flowers had not opened. In the fifth week fertilisation was over and grain formation had started. The intensity of infection was proportional to the number of flowers open at the time of spraying.

Pathogenicity trials.—Barger (1931) has given a long list of graminaceous hosts for *Claviceps purpurea*. Atanasoff (1920) has however stated that this fungus exhibited physiologic specialization and the form on one host might not pass on to all the other hosts. In order to determine the probable host range of this fungus on the Nilgiris artificial inoculations were carried out on the following plants, *viz.*, *Avena sativa* L., *Amphilophis foulkesii* C. Fisch., *Anthoxanthum odoratum* L., *Cymbopogon polyneuros* Stapf., *Chrysopogon zeylanicus* Thw., *Cynodon dactylon* Pers., *Digitaria marginata* Link., *Glyceria fluitans* R. Br., *Hordeum hexastichon* L., *Panicum miliare* Lamk., *Paspalum dilatatum* Poir., *Pennisetum clandestinum* Hochst., *Sporobolus indicus* R. Br., *Tripogon bromoides* Roth. *Ischæmum aristatum* L., *Lolium temulentum* L., *Oplismenus compositus* Beauv. and *Themeda triandra* Forsk. The results were in the negative. But few instances of positive infection were obtained after repeated inoculations on *Triticum vulgare* Vill., *Avenastrum asperum* C. Fisch., and *Vulpia myuros* Gmel. Out of 200 ears of wheat inoculated, infection was observed on two ears. Three out of 25 ears of *Avenastrum asperum* and 8 out of 30 ears of *Vulpia* developed small sclerotia.

Alkaloid content of ergot.—The usefulness of ergot depends on its alkaloid content. The sclerotia produced in the first year were assayed by the Research Officer, Madras Medical College, and the quality of the ergot was pronounced to be good (Thomas, etc., 1942). Assay of one sample by Dr. Mukerji (1943) showed that the alkaloid content calculated as ergotoxine was in the neighbourhood of 0·13 per cent. Meanwhile the B. P. standard of raw ergot had been raised from 0·05 to 0·2 per cent. of alkaloids. This change necessitated the improvement of the quality of the Nilgiri produce.

One of the methods of doing this was by selection of sclerotia having high alkaloid content and producing cultures of the fungus from such selected sclerotia. The use of these cultures for inoculation led to the stepping up of the alkaloid content of the new crop of ergot (Bekesey, 1940). Sclerotia were cut into two halves. One half was used for the assay and the other utilised for the isolation of the fungus if found to have high alkaloid content. The repetition of this process year after year has led to considerable improvement of the alkaloid content of Nilgiris ergot. The results are shown hereunder.

TABLE IV

Improvement in the alkaloid content of ergot by selection

	Average alkaloid content of bulk produce per 100 gm. of ergot		Highest alkaloid content of individual sclerotia per 100 gm. of ergot (as ergotoxine)
	Ergotoxine	Ergometrine	
1944	190 mgm.	8 mgm.	400 mgm.
1945	300		580
1946	391	91	920
1947	470		1190

The analyses of the bulk produce of 1944 and 1946 were carried out by Dr. Mukerji. The improvement of quality by continuous selection is thus shown to be practicable. The improved cultures were employed for large-scale production.

The influence of the application of manures to the rye crop on the yield and quality of the ergot produced was investigated. The rye was raised on a field which had been previously cropped with potato. Individual plots were manured with bone meal, farmyard manure, fish guano, groundnut cake and ammonium sulphate respectively at two levels. The sowings were made in April 1944. The yield of sclerotia was recorded. The ergot from each plot was analysed separately and the alkaloid content determined. The results showed that there was no significant difference between the treatments, either in yield or in alkaloid content. The figures for yield and alkaloid content were as given in Table V.

TABLE V

Manuring and yield and quality of ergot

Treatment	Average yield of ergot per plot gm.	Alkaloid content per 100 gm. of ergot mgm.
FYM. (5 tons per acre) ..	117	220
FYM. (10 do) ..	108	210
Fish guano (1,200 lb. do) ..	121	190
do (600 do) ..	111	200
Bone meal (1.000 lb. do) ..	108	230
do (2,000 lb. do) ..	117	200
G. N. cake (1,000 lb. do) ..	119	180
do (2,000 lb. do) ..	113	200
Amm. sulphate (1 cwt. do) ..	120	220
do (2 cwt. do) ..	117	200
Control ..	110	200

FYM. = Farmyard manure. G.N. cake = Groundnut cake.

GENERAL

A scheme financed by the Madras Government for the large-scale production of ergot has been in operation on the Nilgiris from 1944. These results have been of considerable importance in the selection of the venue for large-scale production of ergot and in deciding the varieties of rye to be grown. During the five years that the scheme has been in progress it has been realised that the most important factor is the synchronisation of the time of flowering with the prevalence of spells of misty weather or light rains. In years when bright weather was experienced during the flowering period with few rainy days (*e.g.*, in 1948) the production of ergot was very disappointing. The yield of ergot has varied according to the attention bestowed on the various operations by the growers. In Sholada (Nilgiris) one farmer was able to harvest 328 pounds of dried ergot from an area of three acres.

Fresh ergot is usually preferred for processing into ergot preparations as it has been reported that it deteriorates in storage with lapse of time. There was occasion to keep in storage over 1,000 pounds of ergot for 18 months. The dry ergot had been packed in paper-lined plywood chests, nailed down and covered over with jute gunnies. The lot had been analysed before storage and the average alkaloid content was 0·39 per cent. After a period of 18 months the stock was opened. The sclerotia were quite normal and no evidence of insect damage or mouldiness was noticed. Samples were taken out and again analysed. The alkaloid content was found to be varying between 0·35 and 0·37 per cent. This was evidenc

enough to show that when well preserved the deterioration of ergot is not significant.

Summary

Large-scale production of ergot has been in progress, on the Nilgiris, under the auspices of the Madras Government. Rye grows well only above 5,600 ft. The crop sown in April gives highest yield of ergot. Six to eight sprayings are necessary for maximum infection. The larger number of rainy days during the flowering period favours infection. Manures do not have any influence in yield or quality of ergot. Australian black winter rye and local rye are suited for large-scale production of ergot. The pathogenicity trials are described. The quality of the ergot was improved by selection of sclerotia containing a high percentage of alkaloids for obtaining cultures. The alkaloid content of the Nilgiri ergot was raised from 0·19 per cent. to 0·40 per cent.

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STUDIES IN SAMPLING TECHNIQUE

Part III. Estimation of Mite-Incidence in Sugarcane

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I. Introduction

The attack of mite *Paratetranychus indicus* Hirst on sugarcane (Khanna and Ramnathan, 1947) has been on the increase in Bihar and in view of the fact that it considerably reduces the effective functioning area of the leaf surface by innumerable webs built on the lower surface and consequently arrests growth, need was felt to determine accurately the magnitude of attack of the pest. The present paper deals with the sampling requirements for such an estimate.

The incidence of mite has been defined as the number of puparia per square inch of the leaf area and its estimation therefore requires the determination of the area of every affected leaf and the counting of the number of puparia in each. Obviously, it becomes too huge a task even if it is to be carried out for a very few clumps. The problem thus is to find out how many fewer clumps, how many fewer canes in a clump and how many fewer leaves in a cane may enable us to estimate the incidence within limits of desired accuracy. Results of similar sampling studies have already been reported (Khanna, *et al.*, 1948) in connection with whitefly.

II. Material

The data have been obtained from the complete enumeration of 3 fields each of about 1/40th acre in size (60′ × 18′) infested with mite. There were 6 rows in each and each row was divided into units of 3′ to facilitate the sampling work. The clumps in each unit, the canes in each clump and the dry and green leaves (affected and healthy) in each cane have been systematically numbered and puparia counted in respect of each affected leaf. The maximum breadth and length of the leaves have also been recorded along with the puparia counts.

The leaf area has been calculated by multiplying the product of the maximum length and breadth of the leaf by 0·7 according to the formula established before (Khanna, 1935) and the puparia per square inch have been obtained by dividing the total number of puparia in the leaf by its area.

III. Structure of the Variance

The problem may be considered as one of four-fold nested sampling with units, clumps, canes and leaves as the zones of the successive orders. The variance of the mean from nested sampling is a function of the different zone variances, the estimation of which is the prerequisite for sampling studies.

The formula for the variance of a mean in a four-fold nested sampling, where the variates are equal in number from unit to unit in a zone, is given by

$$V(x\ldots.) = \frac{\sigma_1^2}{t} + \frac{\sigma_2^2}{n} + \frac{\sigma_3^2}{m} + \frac{\sigma_4^2}{p},$$

where t, n, m, p represent the number of variates in the successive zones and σ_1^2, σ_2^2, σ_3^2 and σ_4^2 the estimates of the variances in the respective zones. If V_1, V_2, V_3 and V_4 denote the different variances as obtained in the usual analysis of variance, the estimates of the zone variances will be obtained as follows:

V_4 will be the unbiased estimate of σ_4^2

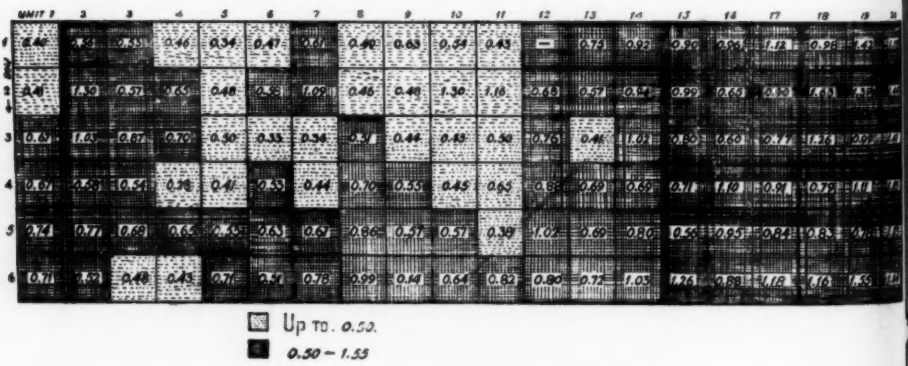
V_3 ,, ,, $\frac{p}{m}\sigma_3^2 + \sigma_4^2$

V_2 ,, ,, $\frac{p}{n}\sigma_2^2 + \frac{p}{m}\sigma_3^2 + \sigma_4^2$

V_1 ,, ,, $\frac{p}{t}\sigma_1^2 + \frac{p}{n}\sigma_2^2 + \frac{p}{m}\sigma_3^2 + \sigma_4^2$.

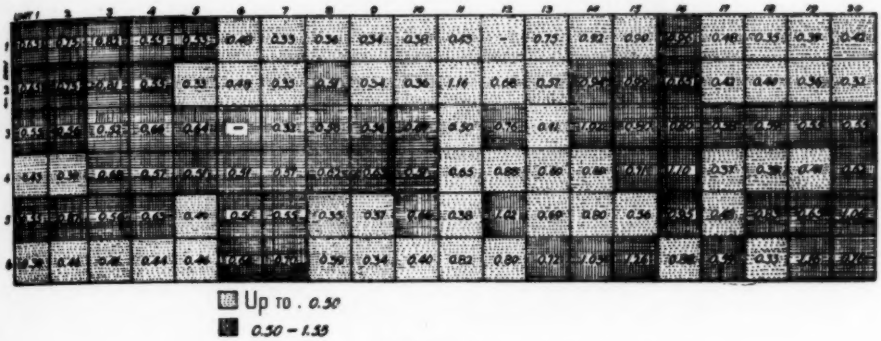
Division into patches and the sampling procedure

Figs. 1, 2 and 3 show the formation of patches in the three plots. The unitwise incidences have also been indicated. Medium and low infestations have been defined as follows:



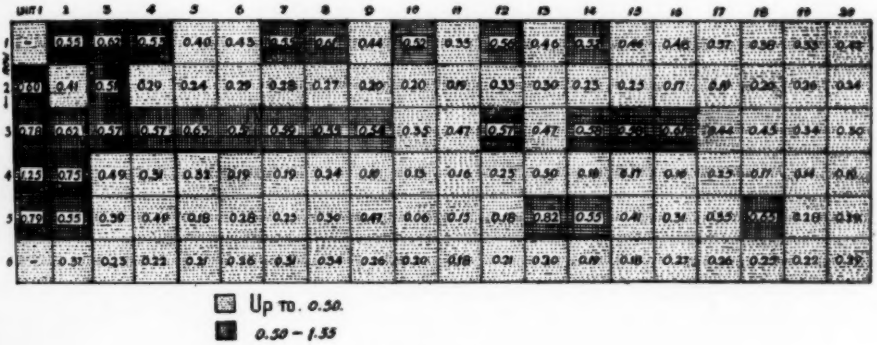
Plot No. 1

Text-Fig. 1. Units shown according to the intensity of incidence *Puparia per sqr. inch of Leaf Area*



PLOT NO. 2

TEXT-FIG. 2. Units shown according to the intensity of incidence
Puparia per sqr. inch of Leaf Area



PLOT NO. 3

TEXT-FIG. 3. Units shown according to the intensity of incidence
Puparia per sqr. inch of Leaf Area

Classes	*Range of incidence*
I. Low	up to 0·50 per square inch
II. Mild	0·50–1·55 per square inch

It will be noticed in the abovementioned diagrams that plot (I) has mild infestation while plot III shows low infestation. In the former 77·5% of the units fall under mild infestation and in the latter low infestation is shown by 75% of the units. Plot II has nearly an equal number of mild and low infested units and exhibits a large patch of mild infestation.

Samples have been drawn separately from the two types of infestation in the three plots. 10 units have been selected at random from each type of infestation as the first order zones and for the remaining zones two clumps

per unit, two canes per clump and five leaves per cane have been selected at random at each stage. One more analysis has been done where 10 units have been selected at random from the whole plot irrespective of the patches. This will be referred to as the pooled analysis. Selection of clumps, canes and leaves has been made in the same manner for the pooled analysis as in the patches. The structure of the analysis of variance is indicated below:

Due to	*D.F.*
Between units	.. 9 (V_1)
Between clumps (within units)	.. 10 (V_2)
Between canes (within clumps)	.. 20 (V_3)
Between leaves (within canes)	.. 160 (V_4)
Total	.. 199

The ultimate variate is the number of puparia per square inch which is a small figure to warrant the necessity of the square root transformation after adding 0·50 to each.

IV. Discussion of Results

The results of the analysis of variance have been shown in Table I. The standard errors have been shown as percentages of the mean. It will be noticed that the standard errors in all the plots have been considerably small being less than 2% in the low patches of plots II and III and less than

TABLE I. *Showing the estimates of variances due to different zones*

Plot No.		Mean–S. E.	*m* expressed as % of mean	Actual				Expressed as % of the total			
				σ_1^2	σ_2^2	σ_3^2	σ_4^2	σ_1^2	σ_2^2	σ_3^2	σ_4^2
1	2	3	4	5	6	7	8	9	10	11	12
I	Mild	1·15–0·0313	2·72	0·0056	0·0000	0·0126	0·0397	9·67	0·00	21·76	68·57
	Low	1·00–0·0251	2·52	0·0000	0·0093	0·0033	0·0159	0·00	32·63	11·58	55·79
	Pooled	1·02–0·0236	2·31	0·0023	0·0017	0·0055	0·0194	7·96	5·88	19·03	67·13
II	Mild	1·05–0·0234	2·23	0·0000	0·0072	0·0029	0·0228	0·00	21·88	8·82	69·30
	Low	0·93–0·0136	1·46	0·0007	0·0006	0·0002	0·0168	3·83	3·28	1·09	91·80
	Pooled	0·97–0·0171	1·75	0·0016	0·0000	0·0003	0·0244	6·08	0·00	1·14	92·78
III	Mild	0·99–0·0394	3·85	0·0121	0·0000	0·0053	0·0232	29·81	0·00	13·05	57·14
	Low	0·87–0·0126	1·45	0·0007	0·0000	0·0026	0·0030	11·11	0·00	41·27	47·62
	Pooled	0·88–0·0125	1·42	0·0012	0·0000	0·0008	0·0053	16·44	0·00	10·96	72·56

σ_1^2 is the variance due to units,
σ_2^2 ,, ,, clumps,
σ_3^2 ,, ,, canes,
and σ_4^2 ,, ,, leaves.

3% in all the other patches except in the mild patch of plot III, where it is 3·85%. It may further be observed that the standard errors under the low patches have been in general lower than those under the mild patch.

The estimates of variances due to the different zones have also been shown in Table I. The last four columns give these variances expressed as percentages of the total. It will be noticed that the leaves have taken the largest percentage of variation ranging from 47·62% to 91·80% in the different patches of the three plots. If the mean square due to a particular hierarchy happens to be less than that in the immediately lower one, the variation due to that hierarchy has been taken as zero and with this definition σ_1^2 and σ_2^2 have come out to be zeros in 2 and 3 cases respectively. σ_1^2 is small also in the remaining patches except in the mild patches of plot III. σ_2^2 is only 3·28% in the low patch of plot II, where the variation due to leaves has been the maximum, being 91·80% and consequently the variations due to the other hierarchies have been small. The variations due to canes range from 1·09% to 41·27%. The magnitude of leaf variations indicates that the totality of leaves is the predominant factor in the effective reduction of the error. The results of pooled analysis have also been shown in the same table for comparison with the patch analysis of the respective plots. From a study of the values of the estimates of the zone variances, it will be seen that the results of mild patch of plot I and low patches of plots II and III agree very closely with those of their respective pooled analyses.

Sample size to be recommended

Different combinations of units × clumps × canes × leaves with hypothetical sample sizes have been worked out keeping the error margin as 5% of the mean. The results have been set out in Table II. Scrutiny of the relative magnitudes of the zone variances in Table I has revealed that sampling may be confined to leaves alone in the low patches of plots II and III. In other words, only the leaf variation in these cases is important and nested sampling is not essential. Standard errors in these patches have been 1·45% and hence for sampling purposes where greater accuracy is required the error margin may as well be fixed at the 2½% level.

A study of Table II reveals that varied sampling sizes are required for each of the two patches of the three plots. The last column of the table gives the total number of leaves to be included in the sample. For mild and low patches, the largest sample sizes have been recorded in plots III and I respectively. To be on the safe side, the largest sample sizes under

TABLE II. *Showing the hypothetical combinations of units, clumps, canes and leaves to keep the error to the level of 5% and 2½% of the mean*

1	Unit 2	Clump 3	Cane 4	Leaf 5	Total No. of leaves 6
5% *Plot* I					
	5	1	1	Impossible	
	10	1	1	3	30
Mild ..	5	1	2	3	30
	10	1	2	1	20
	5	2	1	3	30
	10	2	1	1	20
	5	1	1	Impossible	
	10	1	1	2	20
Low ..	5	1	2	5	50
	10	1	2	1	20
	5	2	1	2	20
	10	2	1	1	20
	5	3	1	1	15
5% *Plot* II					
	5	1	1	6	30
Mild ..	10	1	1	2	20
	5	1	2	1	10
	5	2	1	1	10
	5	1	1	2	10
Low ..	10	1	1	1	10
	5	1	2	1	10
	5	2	1	1	10
5% *Plot* III					
	10	1	1	4	40
	10	1	2	2	40
Mild ..	10	2	1	2	40
	10	2	2	1	40
Low ..	5	1	1	1	5
	10	1	1	1	10
2½% *Plot* II					
	10	1	1	4	40
Low ..	10	1	2	2	40
	10	2	1	2	40
	10	2	2	1	40
2½% *Plot* III					
	5	1	1	2	10
Low ..	10	1	1	1	10
	5	2	1	1	10

the different hypothetical combinations may be recommended and even then the sample sizes as indicated below come within the practicable reach.

	Units	Clumps	Canes	Leaves	Total
Mild ..	10	1	1	4	40
	10	1	2	2	40
	10	2	1	2	40
	10	2	2	1	40
Low ..	5	3	1	1	15

Similar hypothetical sample sizes with different combinations of units × clumps × canes × leaves have also been worked out for the pooled analysis, keeping the error margin as 5% of the mean. The results have been shown in Table III. It is noticed that the sample sizes are not above

TABLE III. *Showing the hypothetical combinations of units, clumps, canes and leaves to keep the error to the level of 5% Pooled Analysis*

Units	Clumps	Canes	Leaf	Total No. of leaves
Plot I				
10	1	1	2	20
5	1	1	6	30
10	2	1	1	20
5	2	1	2	20
10	1	2	1	20
5	1	2	2	20
10	2	2	1	40
5	2	2	1	20
Plot II				
10	1	1	2	20
5	1	1	3	15
10	2	1	1	20
5	2	1	2	20
10	1	2	1	20
5	1	2	2	20
10	2	2	1	40
5	2	2	1	40
Plot III				
10	1	1	1	10
5	1	1	1	5
10	2	1	1	20
5	2	1	1	10
10	1	2	1	20
5	1	2	1	10
10	2	2	1	40
5	2	2	1	20

40 leaves as recommended for the mild patch. It can, therefore, be argued that when the incidence is either mild or mostly mild (representing the pooled patch), 40 leaves selected at random in the manner mentioned above would provide a satisfactory estimate of the incidence.

The table given below shows the means (without transformation) for the whole patch as well as for the samples with which analyses have been done. Samples have also been drawn at random from the patches in acccordance with the recommendations made above and the respective estimated incidences have been shown in the third column.

	Whole patch mean	Mean of the samples with which analyses were done	Mean with recommended sample sizes
Plot I Low ..	0·43	0·49	0·46
Mild ..	0·83	0·87	0·81
Plot II Low ..	0·41	0·39	0·41
Mild ..	0·62	0·63	0·59
Plot III Low ..	0·24	0·27	0·23
Mild ..	0·49	0·53	0·51

It is noticed in the above table that there is practically no difference between the means of the recommended samples and their respective patch means, thus showing the adequacy of the size of samples recommended for estimating the incidence.

V. Summary of Results

1. An attempt has been made to determine the adequate sample size for estimating the mite incidence.

2. It has been observed that incidence varies in degrees of intensity even in a plot of 1/40th acre and sampling requirements are different for patches of varying degrees of intensity.

3. Scrutiny of the variations revealed that in case of low incidence (*i.e.*, where the number of puparia per square inch is below 0·50), nested sampling is not necessary. Sampling may be done on the basis of leaves alone. In such a case 15 random leaves in a plot of 1/40th acre would be sufficient to bring down the error of estimation well within 5% of the mean.

4. For mild infestation (*i.e.*, where the number of puparia per square inch is between 0·50 and 1·55), 40 leaves selected at random from 10 units of 3′ each would be sufficient for the estimation of the incidence in a plot

of 1/40th acre within an error of 5%. The leaves may be distributed between the units, clumps, and canes in the following manner:

Units	Clumps	Canes	Leaves	Total
10	1	1	4	40
10	1	2	2	40
10	2	1	2	40
10	2	2	1	40

Acknowledgement

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PROPAGATION OF *BANGLA* VARIETY OF *PAN* (*PIPER BETLE*) IN THE CENTRAL PROVINCES

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Asthana and Mahmud[1] have worked out the economics of 'seeds' or cuttings of *Kapuri* variety of *pan* (Piper betle) and have shown that it is most economical to take only the topmost three cuttings from each vine for 'seed' purposes. Experiments similar to those described by them were carried out with the *bangla* variety of *pan* at Ramtek in 1943. The data obtained from these experiments are given below.

In 1943 the vines of *bangla* variety for 'seed' purposes were available at an average rate of Rupees thirty-two per thousand. At Ramtek and other places in the southern and eastern districts in the Province cuttings are planted in pairs at the rate of 1,40,000 per acre. The cuttings for the experiments were taken from vines two years old. Each vine was cut from tip to base into twelve pieces, each piece consisting of five internodes and measuring from 12 to 15 inches in length. In the text these pieces will be referred to in order of succession from tip downwards as 1st, 2nd,.....and 12th cuttings respectively. The 1st to 4th cuttings had three leaves each, 5th and 6th cuttings had two leaves each and 7th to 12th cuttings had no leaves on them. The experiment with these cuttings was laid on randomised basis and was replicated four times. As the susceptibility of the different types of cuttings to foot-rot was to be tested, no fungicidal treatment was given. The soil was manured in all cases with linseed oilcake-flour once every month from February to September at the rate of 250 lbs. per acre per application.

The cuttings were sown on the 1st of September 1943. In the case of 1st to 4th cuttings germination started in the 3rd week and continued till the 6th week, while in the case of 5th to 12th cuttings germination started in the 2nd week and continued till the 4th week. Most of the 1st to 4th cuttings germinated in the 4th week, while most of the 5th to 12th cuttings germinated in the 3rd week. The germination in 1st to 3rd cuttings was slightly lower than in 4th to 12th cuttings. Due to poor development of the root system a large number of 7th to 12th cuttings did not survive longer than two months after germination. The incidence of foot-rot

(caused by *Phytophthora parasitica* var. *piperina* Dast.) was also higher in 7th to 12th cuttings than in 1st to 6th ones (Table I).

TABLE I

Mortality due to poor root system and foot-rot

Cutting No.	No. of cuttings germinated out of 100 planted	No. of cuttings died within 3 months due to poor root system	No. of vines died during 12 months due to foot-rot	Percentage of mortality due to poor root system and foot-rot
1st ..	92	..	14	15·2
2nd ..	96	..	11	11·4
3rd ..	96	..	17	17·7
4th ..	99	..	13	13·1
5th ..	98	..	19	19·3
6th ..	100	..	12	12·0
7th ..	100	11	36	47·0
8th ..	97	28	52	82·4
9th ..	99	27	72	100·0
10th ..	100	23	71	94·0
11th ..	94	41	49	95·7
12th ..	98	38	60	100·0

The linear growth of the *pan* vines is shown in Table II. The rate of growth was highest in the 1st cutting and poorest in the 10th, 11th and 12th cuttings.

TABLE II

Average linear growth of the vines

Cutting No.	Dates of Measurement			
	1-12-1943	1-3-1944	1-6-1944	1-9-1944
1st ..	0′-9″	2′-7″	5′-7″	9′-9″
2nd ..	0′-8″	2′-7″	5′-1″	9′-1″
3rd ..	0′-9″	2′-7″	5′-1″	9′-1″
4th ..	0′-7″	2′-1″	4′-7″	8′-11″
5th ..	0′-6″	2′-0″	4′-7″	8′-11″
6th ..	0′-6″	2′-0″	4′-1″	8′-1″
7th ..	0′-6″	1′-7″	4′-0″	7′-4″
8th ..	0′-6″	1′-6″	3′-1″	6′-2″
9th ..	0′-6″	1′-6″	2′-0″	3′-1″
10th ..	0′-6″	1′-3″	2′-0″	2′-9″
11th ..	0′-6″	1′-3″	2′-0″	2′-9″
12th ..	0′-6″	1′-3″	2′-0″	2′-9″

The average yield of *pan* leaves per plant per annum are given in Table III. In 1943 *bade pan* (large leaves) and *Khilli* (small leaves) were sold respectively at an average rate of 1,500 and 8,000 per rupee. *Chote*

TABLE III

Average yield of pan *leaves per plant per annum*

Cutting No.	Total yield per plant per annum					
	Bade Pan		Khilli		Chote Khilli	
	Total No.	Percentage	Total No.	Percentage	Total No.	Percentage
1st ..	51	82·2	11	17·7	..	..
2nd ..	49	79·0	13	20·9	..	..
3rd ..	49	80·3	12	19·6	..	..
4th ..	40	72·7	15	27·2	..	..
5th ..	31	58·4	22	41·5	..	..
6th ..	21	43·8	27	56·2	..	..
7th ..	11	24·4	34	75·5	..	..
8th ..	6	16·6	30	83·3	..	..
9th ..	..	..	15	42·8	20	57·1
10th ..	..	..	..	..	27	100
11th ..	..	..	..	..	29	100
12th ..	..	..	..	..	24	100

khilli (very small leaves) have no market value. Yield of *bade pan* was highest in the 1st, 2nd, 3rd and 4th cuttings, low in the 5th cutting, lower in the 6th and 7th cuttings, least in the 8th cutting and nil in the 9th to 12th cuttings. 6th to 8th cuttings yielded more of *Khilli* than *bade pan*, while the 9th cutting yielded only *khilli* and *chote khilli*. 10th, 11th and 12th cuttings yielded only *chote khilli*.

The yield of *pan* leaves and income from each type of cutting for the first year and for the duration of five years of a *pan* garden are given in Table IV. As *pan* cuttings for plantation are not available ready-made, they have to be made out of vines purchased for 'seed' purposes. After taking the required number of cuttings from a vine, the rest of it is discarded though the leaves from it are picked and sold. If only one 'seed' is taken from each vine the cost of 'seeds' will amount to Rs. 3,428 per acre. The cost of construction of a bareja (*pan* garden) amounts to Rs. 800 per acre. The cost of cultivation amounts to Rs. 1,200 per acre per annum. The total expenditure for five years will amount to Rs. 10,228. The net income from 1st, 2nd, 3rd, 4th, 5th and 6th cuttings will amount to Rs. 14,532, Rs. 13,772, Rs. 13,687, Rs. 9,747, Rs. 6,162 and Rs. 132 respectively. If the 7th or other lower cuttings are planted there will be a definite loss to the cultivator.

The cultivators usually take more than one cutting from each vine for propagation. The cost of such mixed cuttings and the yield of *pan*

TABLE IV

Yield and income from each type of cutting

Cost of cuttings per acre	Rs.	3,428
Cost of construction of a *pan* garden per acre	Rs.	800
Cost of cultivation per acre per annum	,,	1,200
Total expenditure during 1st to 5th year	,,	10,228

Cutting No.	Yield of Bade pan per acre per annum	Income from Bade pan per acre per annum (Rupees)	Yield of Kh lli per acre per annum	Income from Khilli per acre per annum (Rupees)	*Yield of chote Khilli per acre per annum	Total income per acre per annum (Rupees)	Income over expenditure during first year (Rupees)	Total net profit during five years (Rupees)
1st ..	71,40,000	4,760	15,40,000	192		4.952	− 476	+14,532
2nd ..	68,60,000	4,573	18.20.000	227		4,800	− 628	+13,772
3rd ..	68,60,000	4.573	16,80,000	210		4,763	− 645	+13,687
4th ..	56,00,000	3,733	21,00,000	262		3,995	−1,433	+ 9,747
5th ..	43,40,000	2,893	30,80,000	385		3,278	−2,150	+ 6,162
6th ..	29,40,000	1,600	37,80,000	472		2,072	−3,356	+ 132
7th ..	15,40,000	1,026	47,60,000	597		1,623	−3,805	− 2,113
8th ..	8,40,000	560	42,00,000	525		1,085	−4,343	− 4,803
9th ..	..	..	21,00,000	262	28,00,000	262	−5,166	− 8,918
10th ..	..	..	..	..	37,80,000	..	−5,428	−10 228
11th ..	..	..	..	..	40,60,000	..	−5,428	−10,228
12th ..	..	..	..	..	33,60,000	..	−5,428	−10,228

* No income is derived from the yield of chote khilli.

leaves from them would depend on the number of cuttings that are taken from each vine. It will be seen from Table V that the highest income in

TABLE V

Yield and income from mixed cuttings

Cost of construction of a *pan* garden per acre	..	Rs. 800
Cost of cultivation per acre per annum	..	,, 1,200

Cutting No.	Cost of cutting per acre	Total expenditure per acre for the first year (Rupees)	Total income per acre for the first year (Rupees)	Income over expenditure for the first year (Rupees)	Income over expenditure for five years (Rupees)
1st ..	3,428	5,428	4,952	− 466	+14,532
1st and 2nd ..	1,816	3,816	4,876	+1,060	+15,384
1st, 2nd and 3rd ..	1.256	3,256	4,844	+1 588	+15,859
1st to 4th ..	992	2,992	4,631	+1,639	+12,183
1st to 5th ..	796	2,796	4,360	+1,564	+ 8,794
1st to 6th ..	660	2,660	3,978	+1,318	+ 2,900
1st to 7th ..	572	2,572	3.640	+1,068	+ 743
1st to 8th ..	500	2,500	3.309	+ 809	− 1,875
1st to 9th ..	440	2.440	3,042	+ 602	− 5,930
1st to 10th ..	396	2,396	2,742	+ 346	− 7,196
1st to 11th ..	360	2,360	2,417	+ 57	− 7,160
1st to 12th ..	328	2,328	2 203	− 125	− 7,128

five years during the life-time of a *pan* garden from such mixed cuttings is obtained when the 1st, 2nd and 3rd cuttings are taken from each vine for propagation. It will also be seen that if more than 7 cuttings are taken from each vine for propagation it would result in a loss to the cultivators.

The above experiments with *bangla* variety of *pan* have given more or less the same results as were obtained from the experiments carried out by Asthana and Mahmud[1] with *Kapuri* variety of *pan*. Both the sets of experiments referred to above have conclusively shown that if the cultivators would take only the topmost three cuttings for 'seeds' it would not only lessen the initial expenditure incurred on account of 'seeds' and minimise the incidence of foot-rot disease, but it would also give them handsome profits by yielding average net incomes of Rs. 3,171 and Rs. 1,950 for the *bangla* and *Kapuri* varieties of *pan* per acre per annum respectively.

Summary

1. Out of the twelve cuttings from each vine, the first four cuttings took longer period for germination than the rest.

2. Germination in 1st to 3rd cuttings was slightly lower than in 4th to 12th cuttings.

3. Mortality due to poor development of root system was observed in 7th to 12th cuttings.

4. Mortality due to *Phytophthora parasitica* var. *piperina* Dast. was higher in 7th to 12th than in 1st to 6th cuttings.

5. The rate of growth was highest in the 1st cutting.

6. The yield of *pan* leaves was highest in the 1st cutting.

7. The first three cuttings are recommended for 'seed' purposes.

Acknowledgment

I am highly thankful to Dr. R. P. Asthana, M.Sc., Ph.D. (Lond.), D.I.C., F.A.Sc., Mycologist to Government, C.P. and Berar, for giving the necessary facilities for carrying out the experiments described in the paper.

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PARAPETALUS CAUDATUS N. SP., A COPEPOD PARASITIC ON *DUSSUMIERIA ACUTA*, FROM MADRAS

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Parapetalus orientalis Steenstrup and Lutken, *P. occidentalis* Wilson, *P. hirsutus* Bassett-Smith are the only known species of the genus. The present new species parasitic on the clupeid *Dussumieria acuta* differs so markedly from the species of *Parapetalus* described so far that a full description of the parasite and a comparison of all the known species are given in this paper.

Host and Record

Six specimens of the parasite, all female, were found attached to the posterior region of the mouth floor of six different rainbow sardines (*Dussumieria acuta*) caught on the Madras Coast. Nearly 120 fishes of all sizes were examined, hence the percentage of infection appears to be about 5%. Though the six hosts were of different age groups yet the location of the parasite was remarkably uniform. The cephalothorax of the parasite was fixed near the posterior angles of the triangular mouth floor, with the rest of the large flat body extending over the gill arches. The peculiar site of attachment of the parasite probably accounts for its frequently being alive, nearly eight hours after the host fish was landed and is probably also responsible for its large size and flat form.

Definition of the Species.—The carapace is elliptical, less than a fifth of the body in length. The genital segment is more than twice the length of the carapace and widens posteriorly where it ends in two lateral flattened lobes which, however, do not hide the abdomen ventrally. There are no median lobes. Abdomen is only a little longer than the genital segment and has two wide latero-dorsal wings, very nearly the entire length. The fourth or free segment, almost hidden, bears no plates, processes or appendages other than legs which are uniramous and bear a slender seta on the protopod and five spines on the three-jointed exopod. The first leg has a protopod bearing groups of stiff short hooked hairs, and a terminal joint bearing a small spine on its anterior aspect, three aborted setæ on the posterior aspect and five terminal spines.

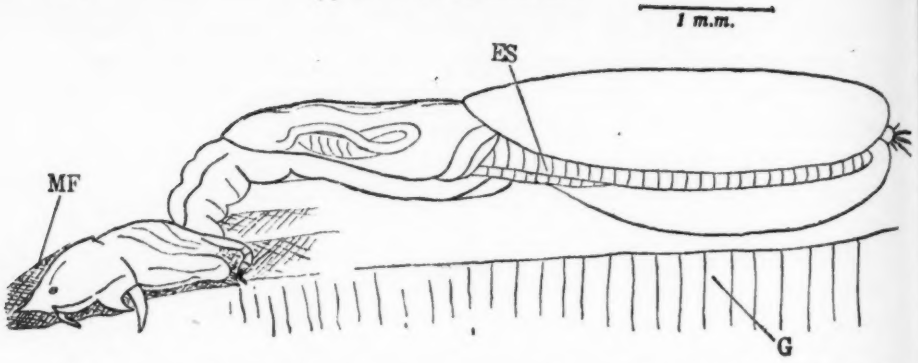

FIG. 1. Lateral view of Parasite attached to mouth floor of fish host. *E. S.*—egg strings of parasite. *G.*—Gills of fish. *M. F.*—mouth floor of fish.

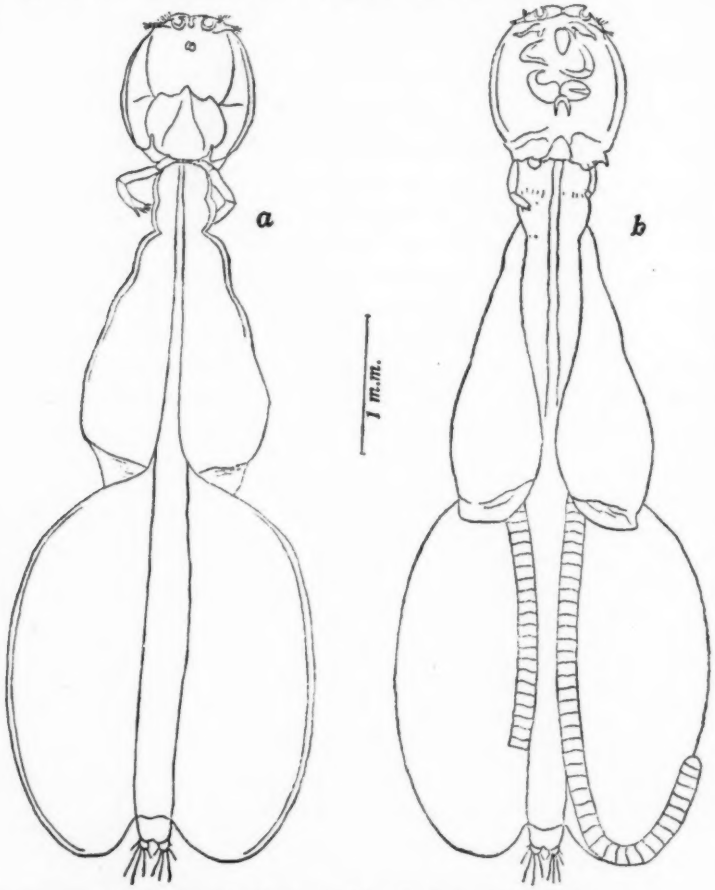

FIG. 2. Dorsal (*a*) and ventral (*b*) views of female *Parapetalus caudatus* n. sp.

Size and Colour.—The parasite with the longest egg-string is 7·4 mm. long and 2·8 mm. across the abdomen where the body is broadest. When alive, the body was pale yellow, the median eyes red, the abdominal extensions whitish and the egg-strings bright yellow.

Body.—The cephalothorax is almost circular being 1·32 mm. long and 1·2 mm. across. Though only about a sixth of the whole body in size, it is the only part attached to the host. The frontal area as well as the lateral margins have a narrow transparent flexible fringe which facilitates adhesion. The lunules on either side of the medially notched frontal plate, are shallow being 0·18 mm. across and 0·4 mm. deep. They are directed ventrally and with their dentate margin, probably, act more by friction than by cupping adhesion characteristic of the lunules of *Caligus*. The disposition of chitin ridges within the lunules, as well as round the edge of the cephalothorax shield, is peculiar and prevents slipping. The cephalothorax being less concave ventrally than in *Caligus*, the appendages seem to be mainly responsible for the tenaceous hold of the parasites. The first three segments of the thorax proper appear united with the cephalon as in all Caliginæ. The fourth (free) segment is small being 0·34 mm. broad and 0·18 mm. long and is invisible ventrally owing to the backward extension of the protopodite of the third leg, and is very nearly hidden dorsally by the backward extension of the dorsal shield and the forward extension of the genital segment behind.

The genital segment is clearly of two parts; an anterior narrow neck-like part, 0·44 mm. long and as broad, constricted in the middle and attached to the cephalothorax at an angle of about 45°, and a hind part which broadens posteriorly and ends in two flattened corners extending backwards ventral to the abdomen. Viewed ventrally these flattened extensions appear to be continuous with the two longitudinal lateral swellings of the genital segment, separated by a groove between. The dorsal side is conspicuously marked by the attachment of the abdomen, a little in front of the lateral corner lobes. The egg-strings are usually straight but when longer than the body they become coiled outwards and forwards.

The abdomen is composed of two segments; the first is greatly elongated and broadened, being (3·4 mm. long and 2·8 mm. broad). Dorsally it is convex and is marked by a median anterior projection and by a posterior notch. In the smaller forms examined the broad lateral wings of the abdomen appear flat, whereas in the mature female forms the wings appear inflated on either side of the median region where the gut runs. These lateral extensions are penetrated by a network of blood channels from two

longitudinal trunks running on either side of the gut. The second segment of the abdomen is much shorter, being one twentieth of the entire length of the abdomen, and occupies the median notch behind. It bears two short rounded anal laminæ; each lamina carries one short slender bristle on either side, and five plumose setæ of which the central three are far longer.

Appendages

The 1*st antenna* is two-jointed. The stout basal joint bears the lunules as well as eleven plumose setæ along the anterior edge and nine spines ventral to them. The distal segment which is club-shaped bears twelve spines.

The 2*nd antenna* is two-jointed and has a single-toothed lobe on its basal joint while the distal segment is long and ends in a powerful hook.

The mouth tube is long, conical and is supported by several rods jointed about the middle of the tube. The labrum and labium are similar semi-circular lobes at the end of the tube. At either corner of the mouth occur two slender processes. The mandible is sickle-shaped, the distal joint bears sixteen teeth, the more proximal ones being smaller than the others.

The 1*st maxilla* is a broad, short, pointed structure bearing two recurved spiny setæ—the vestiges of the palp.

The 2*nd maxilla* is similar but longer and more curved. Its simple spine-like character allies this form to the genus *Caligus*. The seta at its base probably represents the exopod or palp.

The 1*st maxilliped* is three-jointed, the long distal part bearing three sharp curved hooks. The 2nd maxilliped is of a large and powerful build. The basal joint is extremely stout and broad, while the distal segment is a strong sharp curved hook, and is the main organ of attachment to the host. The furca is slightly divergent, straight, and sharp-pronged. The 1*st leg* is uniramous as in *Caligus*. The basal segment is stout, and bears a spine-like seta. The exopod is two-jointed; the proximal joint bears groups of stiff, short, hooked hairs on its hinder edge and is nearly thrice as long as the distal. The distal joint is slender and bears five spiny setæ as well as four short recurved prickles. Of these prickles three on the posterior margin are aborted setæ. The 2*nd leg* is biramous. The protopod is two-jointed, the distal being nearly thrice as large as the basal, which bears a sharp plumose seta. The endopod is three-jointed and bears six plumose setæ on the distal rounded joint and two on the inner aspect of the middle joint. The exopod is also three-jointed and bears on its outer aspect three spines one on each joint, as well as six plumose setæ on the medial side of

the two terminal joints. The 3*rd leg* is much reduced, though it still has the foliaceous biramous form, as in Caligines. The protopodite is large and laminate and extends backward over the short free segment and bears a short slender plumose seta on either side of the median line. The exopod is three-jointed; the basal joint bears a large hook-like spine, the longer middle joint is fringed with hairs along its edges and bears a plumose seta at its inner tip, while the third joint bears three slender spines and three

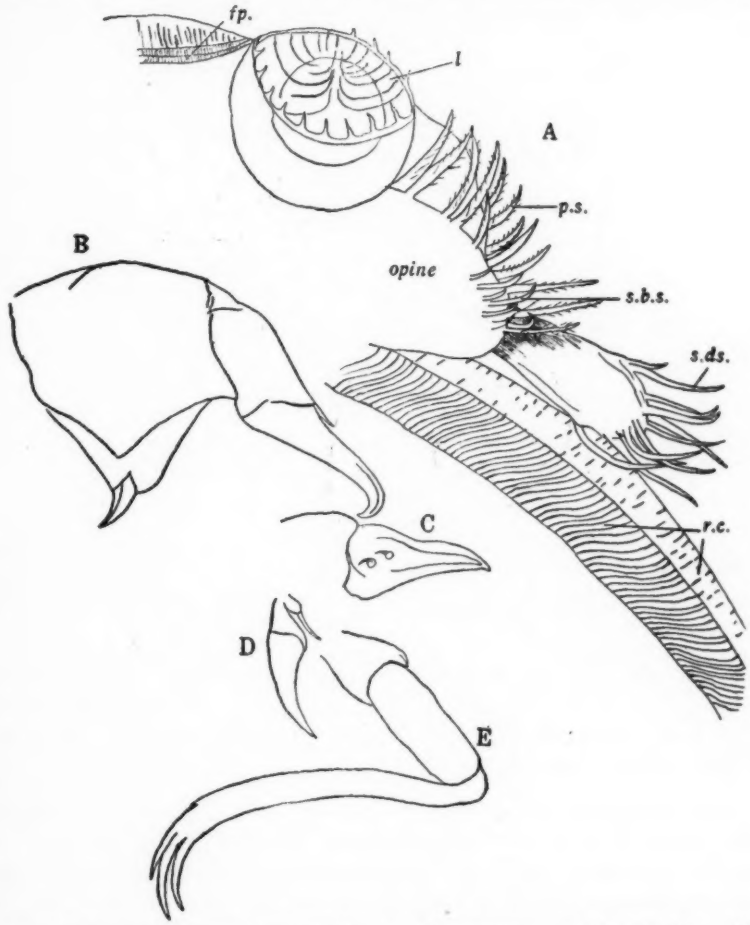

FIG. 3. Antennæ, maxillæ and maxillipede. A. *First Antenna* .*p.f* frontal plate. *l*, lunule. *p. s.* plumose seta. *r. c.* rim of carapace showing flexible fringe and chitinous thickening. *s. b. s.* spines of basal segment. *s.d.s.*- spines of distal segment. B. *Second Antenna* *C.*—First Mexilla. *D.*—Second Maxilla. *E.*—First Maxillipede.

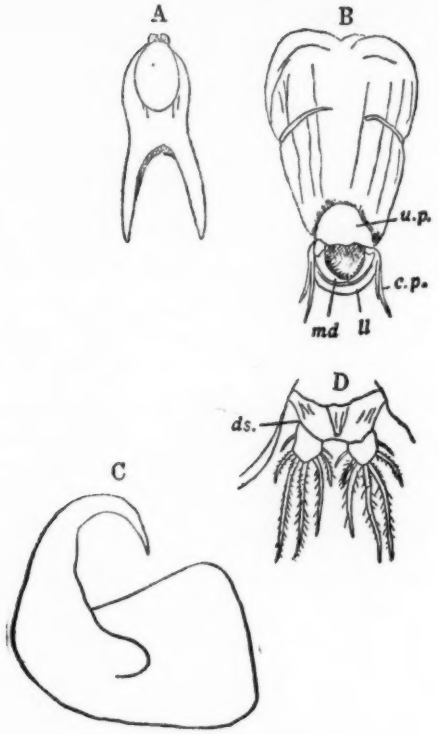

FIG. 4. Furca, mouth tube, II maxillipede and anal laminae. *A*. Furca. *B*. *Mouth tube*, *cp*. corner process. *ll*. lower lip. *Md*. mandible. *Up*. upper iip. *C*. Second maxillipede. *D*. anal laminæ. *ds*. distal segment of abdomen.

plumose setæ. The endopod is two-jointed; the basal joint which is very short and is sunk into a notch of the protopodite, bears a long plumose seta on the inner edge, while the second joint is nearly circular and bears six plumose setæ of varying lengths.

The *4th leg* is uniramous. To the long basal segment is articulated at its outer end a slender seta, probably representing the vestigial ramus. The distal part of the leg is of two joints; the first, longer joint bears at its outer distal tip a sharped curved spine, the terminal joint bears a shorter spine on its outer margin and three at its free tip. There are no vestiges of the 5th and 6th appendages.

The type will be lodged with the Zoological Survey of India, Calcutta.

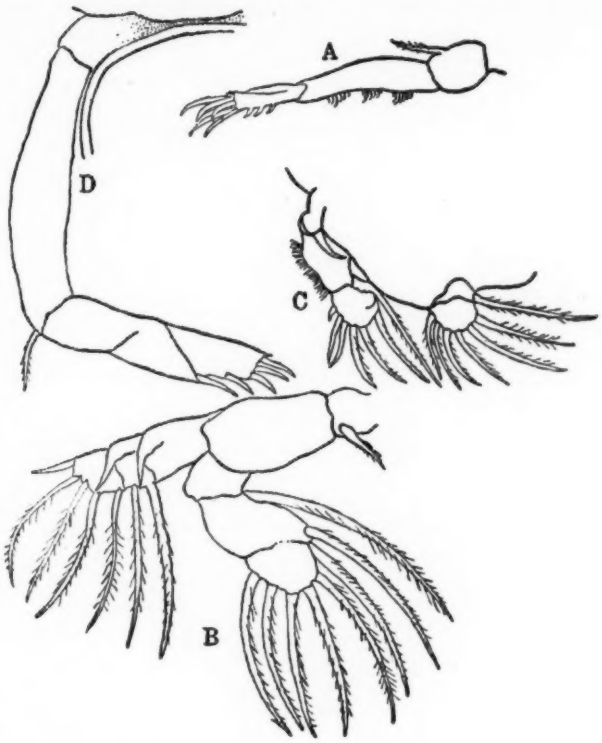

FIG. 5. Posterior appendages. *A* = First leg. *B* = Second leg. *C* = Third leg. *D* = Fourth leg.

TAXONOMIC DISCUSSION

Wilson (1905) in his classification of the Caligidæ places the genus *Parapetalus* of Steenstrup and Lutken, with its type species *P. orientalis*, in the sub-family Caliginæ, which is characterised by the IV segment having no dorsal plates or any appendages other than the IV legs. Within the sub-family, the genus is distinguished from the others by the uniramous character of 1st and 4th legs, the frontal plates being provided with lunules, the 2nd maxillæ being simple and spine-like and both the genital segment and abdomen being winged. A brief comparison of the present form with the other species as given in the following table will show that it belongs to a new species of this genus.

Note on Respiration.—The gill arches of the fish host with the parasite *in situ* were cut out and transferred to fresh sea water and the parasite was observed alive for a few hours. The regular rhythmic movements of the posterior part of the body helping circulation,

	P. caudatus n. sp.	*P. orientalis* Steenstrup and Lutken 1861	*P. hirsutus* Bassett-Smith 1898 redescribed by Wilson 1912	*P. occidentalis* Wilson 1908
Second Maxillæ	Long, curved with a single seta on base		Long, bears two basal setæ	Short and stout, bears two setæ
First leg ..	Protopod bears groups of stiff hooked hairs; distal joint bears a small spine on its anterior aspect, three aborted setæ on its posterior margin and five terminal spines	The second joint bears a spine, the third bears five spines and three plumose setæ	Protopod bears a spur, second joint a spine and the last joint, three spines and three plumose setæ	Protopod bears a spine; distal segment has four spines and three plumose setæ
Fourth leg ..	Protopod long, bears a slender seta and five spines on the two joints of the exopod	Protopod long, three jointed; ramus bears five spines	Protopod long bears a small spine the three joints of ramus bear five spines (Wilson is silent about the clusters of hairs Bassett-Smith noted at the base of spine)	Protopod long, three jointed exopod bears five spines
Carapace ..	Elliptical, less than one-fifth of body length	Orbicular, two-fifths of body length	Elliptical one-third the body length	Ovate, one third the body length
Genital segment	More than twice the length of carapace widens posteriorly and ends in two lateral flattened lobes	Has lateral extensions narrow in front broad behind. Posterior extensions large and hide abdomen ventrally	Three fourths the carpace, widens posteriorly. Posterior lobes not large	As large as carapace, has wide ventrolateral wings curling dorasalwards. Posterior extensions as long as abdomen. There are two median lobes which unite into one after maturity
Abdomen	Longer than genital segment by a fourth. Broadest part of the body. Dorsal surfaee drawn into lateral wings—except for about a twentieth of the length of abdomen	Two thirds the genital segment; produced into wings for about a third of the length	Once and half again as long as genital segment. Wings not up to the end of abdomen	As large has genital segment. Dorsal wings along the entire length

observed in *Caligus savala* Gnanamuthu, were clearly visible. The broad abdomen could be seen swung upwards and downwards through an arc, at intervals of 5 to 15 seconds — the interval being shorter when the host fish was fresher. The large flattened extensions of the abdomen which are traversed by a close network of blood channels, suggest that they have a respiratory function, thus indirectly testifying to the skin being the site of respiratory exchange. The peristaltic movements of the gut were amphidirectional and irregular, as in *Caligus*. Observations of these peristalses, as well as the movements of the rectum, limited as they were to a few hours, however, did not support the possibility of respiration being carried on through the anus.

The author thanks Prof. T. L. Tuxen of Copenhagen University for Steenstrup and Lutken's paper and Miss Dalsgaard of Madras for translating passages from it.

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MORPHOLOGY AND CYTOLOGY OF *POLYSIPHONIA PLATYCARPA* BOERGESEN*

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(With 2 Plates and Text-Figs. 1-109)

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The only species of *Polysiphonia* that has been worked out in detail both as regards its developmental morphology and cytology is *Polysiphonia violacea* (Yamanouchi, 1906, 1906 A). Since then the same species and a few other species of the genus have been worked out by other authors as regards their developmental morphology (Connolly, 1911; Kylin, 1923, 1937 A; Grubb, 1925; Rosenberg, 1933), but the cytological details of these forms were not followed by these workers.

Polysiphonia platycarpa (Boergesen, 1934, pp. 23–25; 1935, pp. 60–62; 1937, p. 349) occurs very commonly in brackish-water in the estuarine region of the river Adyar at Madras and also in the back-waters at Ennur, a place eleven miles north of Madras. Advantage was taken of its common occurrence for a detailed investigation of its developmental morphology and cytology.

The alga grows as an epiphyte on the leaves of *Halpohila ovalis* and *Cymodocea* (Plate VII, Fig. 6). In later stages, it breaks away from its attachment, grows very much in size and floats as large reddish spherical masses in the water. The alga begins to appear about the end of May or the beginning of June and reaches its maximum abundance in September and October, when it is washed away into the sea during the North-East monsoon season.

The alga was fixed both in the field and later on in the laboratory during the twenty-four hours of the day at intervals of one hour each. Most abundant nuclear division stages were found in material fixed between 9 p.m. and 1 a.m. The material was fixed in Flemming's chrom-acetic-osmic fluid (weaker formula) and was left in the fixing fluid for about 10-15 minutes. If the material is left in the fixing fluid for a longer period, it led to the fragmentation of the material. The material after fixation was washed in a few changes of water and passed upwards through the alcohol grades to 70% alcohol. The following procedure suggested by Yamanouchi (1906 A) was also used and was found very suitable. The material was transferred from the fixing fluid directly to 20% alcohol without washing in water. It

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was then passed slowly upwards through the alcohol grades being kept in each grade for a longer time, so that the chromic acid was extracted by the lower grades as completely as possible. Finally, the material was left in 70% alcohol. This procedure was found very convenient as it minimised fragmentation.

The material was also fixed in form-acetic-alcohol with the following formula which is a slight modification of the one given by Westbrook (1928): alcohol 70% in brackish-water—100 c.c., pure formalin—6·5 c.c. and glacial acetic acid—6·5 c.c. The advantage in using this fluid is that the material could be transferred directly to 70% alcohol from the fixing fluid, thus saving a lot of time and also avoiding the chances of fragmentation. Moreover, the material could be left for a longer time in the fixing fluid ensuring a more thorough fixation and hardening.

Material intended for later work was left in 70% alcohol to which one-tenths of its own volume of glycerine was added. The addition of glycerine was found to be an advantage, since it prevented the material from becoming too brittle and from getting easily fragmented on handling.

For imbedding the material in paraffin, the following procedure was adopted. Single specimens from the material in 70% alcohol were taken out by their lower extremity and gently rinsed in 70% alcohol till the filaments were lying more or less parallel to one another and free from entanglements. Then the material was carefully rolled up in a piece of lens-paper cut to the required size. These rolled up pieces were passed upwards through the alcohol and the alcohol-xylol grades. While imbedding, the paper was carefully removed and the material oriented with a pair of hot needles. Sections were cut 4-10 μ thick with a Spencer rotary microtome, stained in iron-alum hæmatoxylin and mounted in neutral canada balsam.

Whole mount preparations of the material also were made after staining it in an 1% solution of eosin or erythrosin in 50% alcohol and mounting in glycerine. These preparations were found to be of much help in confirming and supplementing the observations made on the microtome sections.

Structure of the thallus

The thallus is constructed on the central axis plan as in the other radial members of the Rhodomelaceæ and its growth is by means of an apical cell. The apical cell cuts off segments on its under side. These segments are cut off by walls which are not perpendicular to the longitudinal axis but a little oblique to it, so that one side of the cell that is cut off is broader than the other. Each segment is capable of producing a lateral structure which may

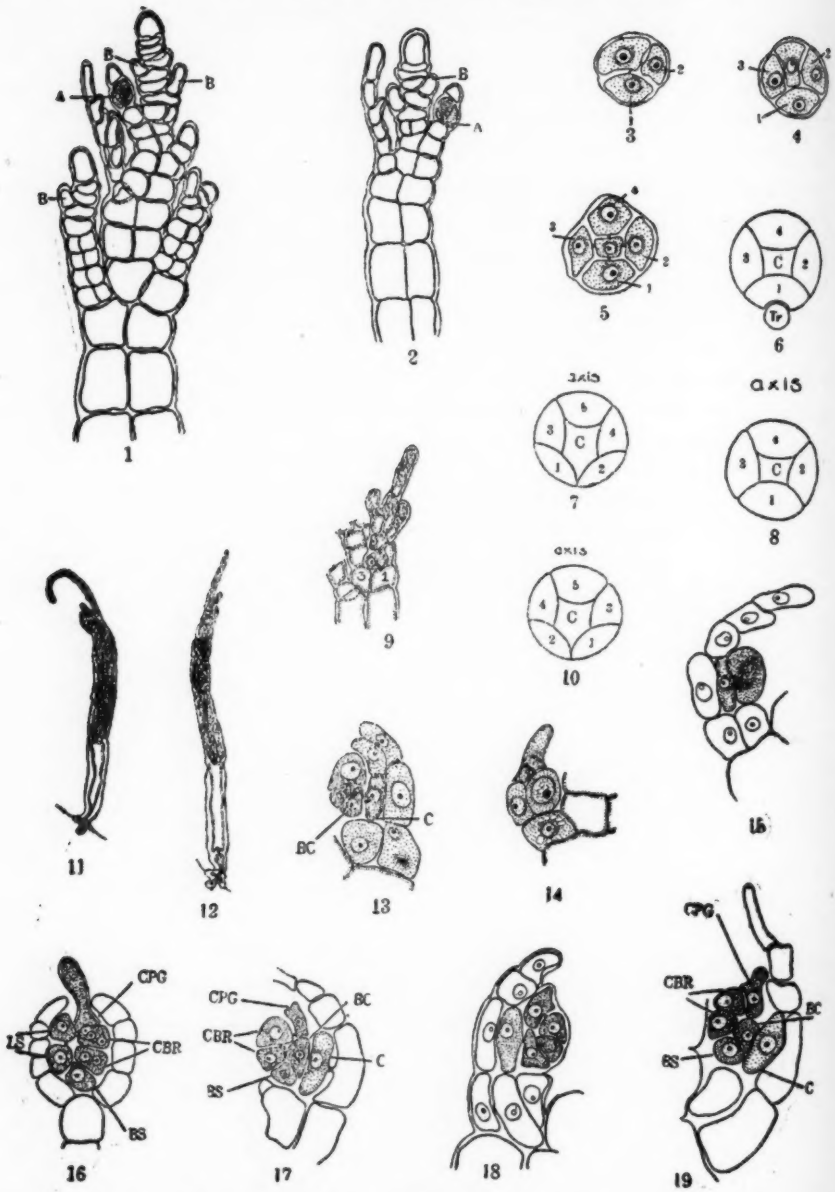
be either a branch or a trichoblast, but all the segments of the main axis do not produce them. The branches or trichoblasts are produced from the uppermost portion of the broader side of the cell from special cells called "Ast Zellen" or branch cells. These branch cells are cut off in the third or fourth segment from the apex.

In the radially constructed Rhodomelaceæ, two types of branch formation are met with. In the first type, the lateral polysiphonous branch is produced by the basal segment of the trichoblast. In the second type, the branches are produced in the place of the trichoblasts themselves. The alga under investigation belongs to the latter type and the lateral appendages are either trichoblasts or branches (*cf.* Boergeseh, 1934, p. 24). The lateral appendages are arranged on the main axis in a left-handed spiral with a divergence of one-fourth, the fourth lateral being placed directly above the first.

Pericentral cell formation

The thallus is polysiphonous and a cross-section shows a single central cell surrounded by four pericentral cells. No cortical cells are formed in this species. The apical portion of the thallus, however, is monosiphonous up to the sixth or seventh segment from the apex. Pericentral cell formation commences only below this level. As a rule, the first formed pericentral cell is cut off immediately below the branch cell of that segment (Text-Fig. 6). The second pericentral cell is cut off to the right of the first pericentral cell and is closely abutting on it (Text-Figs. 3, 6). The third pericentral cell is cut off to the left of the first (Text-Figs. 4, 6), and the fourth is cut off directly opposite to the first (Text-Figs. 5, 6), so that finally we come to have a central cell surrounded by four pericentral cells. The order of formation of the pericentral cells thus follows the general scheme of the structure of the thallus and is in strict conformity with the left-handed spiral arrangement of the lateral appendages. This sequence in the pericentral cell formation is also in agreement with what the previous workers have recorded for other radial members of the Rhodomelaceæ with a left-handed spiral arrangement (Falkenberg, 1901, p. 4; Kylin, 1914; 1923, p. 117; 1937, pp. 136-46; 1937 A, p.1; Rosenberg, 1933, pp. 10-22).

In the younger stages the central and pericentral cells are all of the same size and uninucleate, but later on, as they grow older, they enlarge in size, but not equally. The central cell does not increase in breadth, but gets very much elongated, whereas the pericentral cells, while elongating to the same extent as the central cell, increase very much in width as well. Again the central cell remains uninucleate throughout, whereas the pericentral ones soon become multinucleate. In the central cell, plastids are not evident,



TEXT-FIGS. 1–19. *Polysiphonia platycarpa* Boergs.—Figs. 1–2. Growing portions of cystocarpic plants, showing the apical cell and the arrangement of the trichoblasts and the branches. Figs. 3–5. Serial sections of the main axis showing the sequence of pericentral cell formation;

numbers 1, 2, 3, etc., denote the order of pericentral cell formation. Fig. 6. Schematic representation of a section of the main axis, showing the formation of the pericentral cells ; numbers as in Fig. 5. Figs. 7 and 10. Schematic representation of the sections of the procarpic segment, showing the order of pericentral cell formation ; numbers as in Fig. 5. Fig 8. Schematic representation of a section of a lateral branch, showing the order of formation of the pericentral cells ; numbers as in Fig. 5. Fig. 9. A young trichoblast, showing its structure and position ; 1 and 3 represent the first and the third pericentral cells of the segment. Figs. 11–12. Sporelings, showing the holdfast and the uncorticated basal segments. Figs. 13–15, 17–19. Stages in the development of the procarp. Fig. 16. Procarp before fertilization seen from the front, showing the two lateral sterile cells, the basal sterile cell and the carpogonial branch. *A*, first pericentral cell of the procarpic segment ; *B*, branch cell ; *BC*, bearing cell ; *BS*, basal sterile cell ; *C*, central cell; *CBR*, carpogonial branch ; *CPG*, carpogonium ; *LS*, lateral sterile cells ; *Tr*, trichoblast. (Figs. 1, 2, 9 × 375 ; Figs. 3–5, 13–19 × 600 ; Figs. 11, 12 × 100).

but in the pericentral cells numerous disc-shaped plastids are prominently seen (Text-Fig. 66). The plastids are distributed in the cytoplasm close to the inner and lateral walls of the pericentral cells.

Development of trichoblasts

As already mentioned, the first formed pericentral cell is cut off just below a lateral branch or a trichoblast initial. But, during the later development of the trichoblast, it becomes slightly displaced to one side, so that the trichoblast appears as though arising from between the first and the third pericentral cells (Text-Fig. 9). The trichoblast is richly branched, every cell producing a branch excepting the basal one which remains unelongated and more or less spherical. The branching of the trichoblast also follows the same left-handed spiral arrangement found in the main axis.

Development of lateral branches

The branch cells of the main axis which give rise to the lateral branches divide by walls more or less at right angles to the axis of the newly formed branch. After the formation of a row of cells in this manner, the apical cell of the branch divides by an oblique wall. Thereafter, the further growth of the lateral branch is more or less like that of the main axis. The formation of the pericentral cells in the lateral branch is also quite similar to that of the main axis, and closely follows the left-handed spiral arrangement of the main axis. The first pericentral cell is cut off on the side of the branch farthest from the main axis (Text-Fig. 8). The second and the third pericentral cells are cut off to the right and to the left of the first pericentral cell, respectively, as in the main axis (Text-Fig. 8), and finally the fourth pericentral cell is cut off directly opposite to the first, *i.e.*, on the side nearest to the main axis (Text-Fig. 8). The position of the first two pericentral cells both in the main axis and in the polysiphonous lateral branch in the present

form closely agrees with that of *P. nigrescens* (Kylin, 1923, p. 117; Rosenberg, 1933, pp. 10-11, 14, 15).

Somatic nuclear division

The cells of the thallus in the youngest portions are uninucleate. Later on all excepting the cells of the central siphon become multinucleate. Nuclear division can be easily followed in the apical cells of the main axes and of the branches and also in the cells of the trichoblasts. The resting nucleus is about 3-4 μ in diameter, and shows a very fine reticulum and a conspicuous nucleolus (Text-Fig. 88). During early prophase, the chromosomes are rod-shaped (Text-Fig. 88) and in late prophase, they become shorter (Text-Fig. 89). During metaphase the chromosomes are arranged in an equatorial plate and a distinct spindle could be observed (Text-Figs. 28, 29, 90). The spindle is intranuclear (Text-Fig. 28). Anaphase (Text-Figs. 30, 91) and telophase follow and two daughter-nuclei are organised. An exact count of the chromosomes could not be made on account of the very small size of the nucleus. The chromosome number appears to be about 26 in the male and the female plants and about twice that number in the tetrasporic plants.

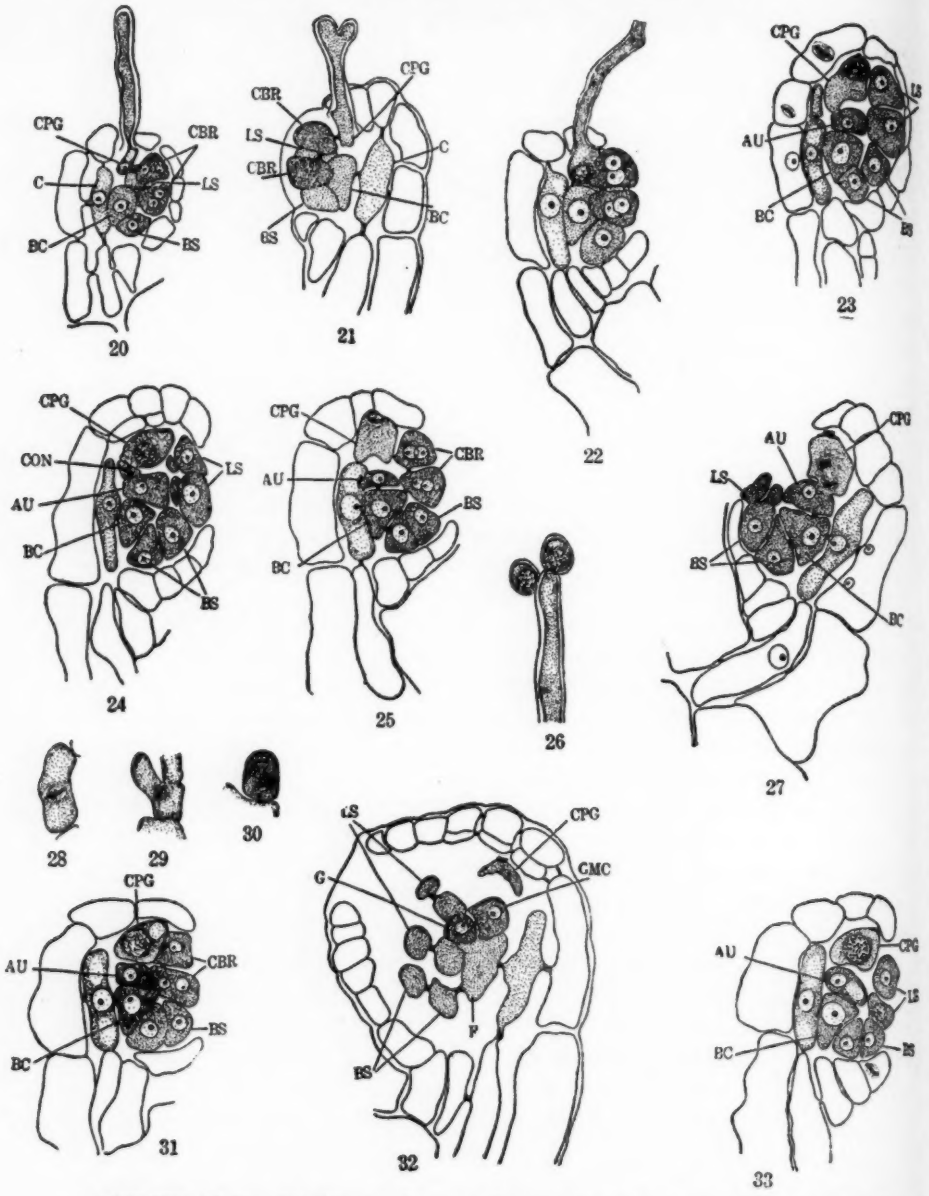
Development of the procarp up to the time of fertilization

The procarp arises on the young trichoblasts very close to the growing apex of the main axis (Text-Figs. 1, 2). Only one procarp is formed on each trichoblast. The procarp is always formed from the second segment from the base of the trichoblast. The trichoblast is monosiphonous to start with. Later on, however, its second segment becomes polysiphonous. Unlike the segments of the main axis it has five pericentral cells instead of four. Soon after, the lowermost segment of the trichoblast also becomes polysiphonous. But this segment resembles the segments of the main axis in having only four pericentral cells. Coming to the formation of the pericentral cells of the second segment (the procarpic segment), the first two pericentral cells are cut off on the side of the trichoblast away from the main axis to the right and to the left, respectively (Text-Figs. 7, 10). There is no definite rule as to the position of the first formed pericentral cell. It is formed either to the right (Text-Figs. 1A, 10) or to the left (Text-Figs. 2A, 7) as the case may be. If the first pericentral cell is cut off to the left, the second pericentral cell is cut off to the right of it and closely abuts on it (Text-Fig. 7). If the first pericentral cell is formed to the right, the second is cut off to the left of it (Text-Fig. 10). The third and the fourth pericentral cells are formed one on each side, the third abutting on the first and the fourth on the second pericentral cell, respectively (Text Figs. 7, 10). Finally the fifth pericentral

cell is cut off on the side nearest to the main axis (Text-Figs. 7, 10). This fifth and the last pericentral cell to be formed is the fertile pericentral cell and gives rise to the procarp.

The fertile pericentral cell (Text-Figs. 15, 38 *PC*) then divides unequally to form a large upper cell and a smaller lower cell (Text-Fig. 39). Of these, the upper is the carpogonial-branch-initial, while the lower is the bearing cell (Text-Fig. 39 *CI*, *BC*). The carpogonial-branch-initial then divides to form a row of three cells (the carpogonial branch) (Text-Figs. 18, 40, 41 *CBR*), the topmost cell of which becomes the carpogonium with a long trichogyne (Text-Figs. 20, 42 *CPG*). Thus we have at this stage a bearing cell on which is situated a carpogonial branch of three cells of which the terminal one is the carpogonium with a trichogyne. The bearing cell and the two carpogonial branch cells are rich in contents, but the carpogonium itself is comparatively poorer in contents. About the time when the carpogonial branch is three-celled, the bearing cell first cuts off a cell towards its base, the *basal sterile cell* (Text-Figs. 17, 19, 41, 42 *BS*) and later on another towards the side (the *lateral sterile cell*). The lateral sterile cell then divides into two to form two lateral sterile cells (Text-Figs. 16, 44 *LS*), whereas the basal sterile cell remains undivided. With the division of the lateral sterile cells into two, the divisions in the procarpic elements come to an end and no further divisions take place until after fertilization, so that, at the time of fertilization, the bearing cell supports (i) a three-celled carpogonial branch, (ii) a lateral sterile branch of two cells, and (iii) a basal sterile branch consisting of a single cell (Text-Figs. 16, 20, 44, 45). The procarp cells are primarily uninucleate, but the two carpogonial branch cells below the carpogonium become binucleate (Text-Figs. 16, 20, 22). In the carpogonium of *P. violacea*, Yamanouchi (1906 A, p. 413, Fig. 104) found two nuclei, one in the sac of the carpogonium, and the other in the trichogyne. In the species under investigation, the writers were unable to find a trichogyne nucleus though numerous carpogonia in all stages of development were very carefully examined. In this respect the writers' observations are in agreement with those of Kylin (1923, p. 118) and Rosenbereg (1933, p. 20) who were unable to find a trichogyne nucleus in *Polysiphonia nigrescens* and in *Polysiphonia Brodiaei*, respectively. Kylin (1914), however, found a trichogyne nucleus in *Rhodomela virgata*.

Several cases of spermatia attached to the trichogyne were observed. The passing of the spermatial nucleus down the trichogyne also was seen in one case (Text-Fig. 22). But the actual fusion of the male and the female nuclei in the carpogonium was not observed. The nucleus of the spermatium when attached to the trichogyne appeared to be in late prophase (Text-Fig.



TEXT-FIGS. 20-33. *Polysiphonia platycarpa* Boergs.—Fig. 20. Procarp just before fertilization seen from the side, showing the bearing cell, the basal sterile cell and the three-celled carpogonial branch ; the lateral sterile cells are shown behind the carpogonial branch. Fig. 21. Pro-

carp with carpogonium showing a forked trichogyne. Fig. 22. Procarp showing two male nuclei passing down the trichogyne. Figs. 23, 25. Procarp after fertilization, showing the fusion nucleus in metaphase ; note in Fig. 23 the open communication between the auxiliary cell and the carpogonium. Fig. 24. Procarp after fertilization, showing the auxiliary cell, the connecting cell, the two basal and the four lateral sterile cells. Fig. 26. Tip of a trichogyne with two spermatia attached to it ; note nuclei of spermatia in late prophase. Fig. 27. Fusion of carpogonium with auxiliary cell ; note the fusion nucleus in late anaphase, and one of the groups of daughter chromosomes inside the canal-like communication between the auxiliary cell and the carpogonium. Figs. 28–30. Metaphase and anaphase of the somatic mitosis in the cystocarpic plant. Figs. 31, 33. Procarp after fertilization, showing the prophases of the first division of the fusion nucleus. Fig. 32. Formation of the gonimoblast ; note the four lateral sterile cells, the two basal sterile cells and the degenerated remnant of the carpogonium above. *AU*, auxiliary cell ; *BC*, bearing cell ; *BS*, basal sterile cells ; *C*, central cell ; *CBR*, carpogonial branch ; *CPG*, carpogonium ; *CON*, connecting cell ; *LS*, lateral sterile cells ; *G*, gonimoblast ; *GMC*, gonimoblast-mother-cell. (Figs. 20–27, 31, 33 × 600 ; Figs. 28–30 × 750 ; Fig. 32 × 535).

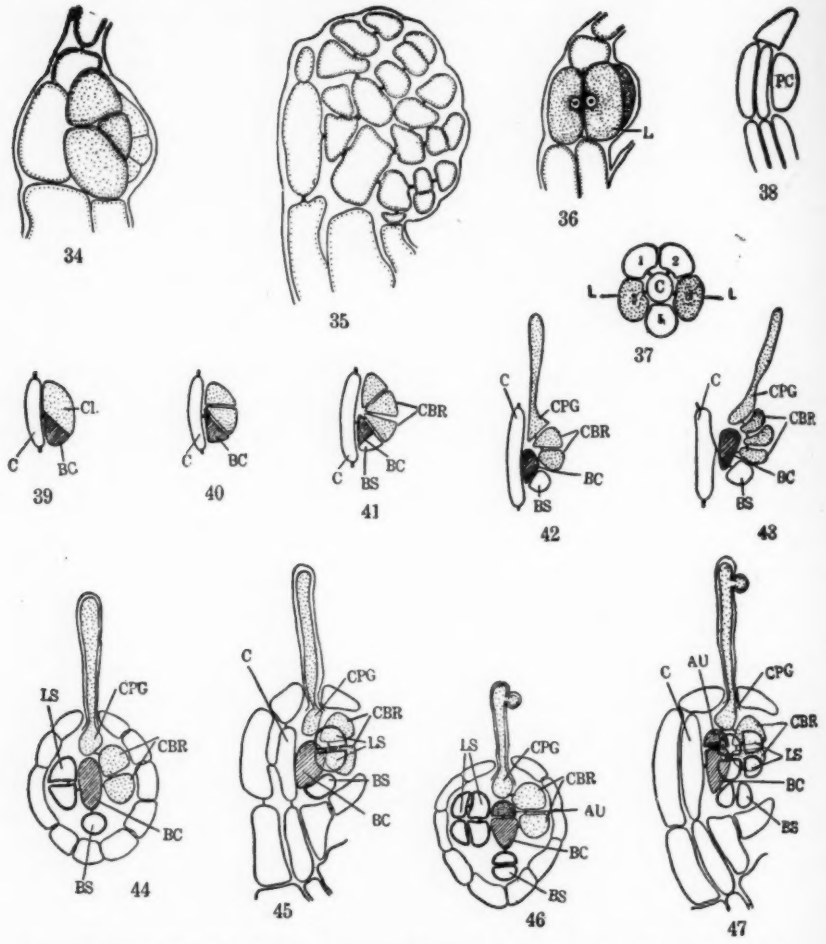
26) and the nucleus passing down the trichogyne also appeared to be in this condition. This is quite in agreement with the observations of Yamanouchi (1906 A, p. 415) in *Polysiphonia violacea* and of Kylin (1923, p. 122) in *Polysiphonia nigrescens.*

Development of the procarp after fertilization

After fertilization the carpogonium begins to enlarge considerably and the trichogyne portion is soon cut off by a wall. The fusion nucleus inside the carpogonium enlarges and begins to divide (Text-Figs. 25, 31, 33). While the division of the fusion nucleus is taking place inside the carpogonium, a series of cell-divisions takes place in the other procarpic elements also. The lateral sterile cells which were two in number before fertilization now divide into four cells; and the basal sterile cell now divides into two (Text-Figs. 24, 46, 47).

Side by side with these divisions, the bearing cell also enlarges and soon cuts off the auxiliary cell at its upper side (Text-Figs. 31, 33 *AU*). The auxiliary cell that is cut off is directly below the carpogonium and is in close proximity to it. The cutting off of the auxiliary cell always takes place only after fertilization. With the formation of the auxiliary cell, and the division of the group of sterile cells, the carpogonial branch cells begin to degenerate.

After the formation of the auxiliary cell, a small cell is cut off from the base of the carpogonium (Text-Fig. 24 *Con*). This small cell corresponds to the *connecting cell* seen in *Ceramium* and *Antithamnion.* Such a connecting cell has been recorded by Oltmanns (1898) in *Dasya,* by Connolly (1911) in *Polysiphonia decipiens* and by Kylin (1923) in *Laurencia pinnatifida.* Kylin (1914; 1923, p. 120), however, did not find any connecting cell either in *Rhodomela virgata* or in *Polysiphonia nigrescens.* The connecting cell fuses with the auxiliary cell and establishes an open canal-like communication

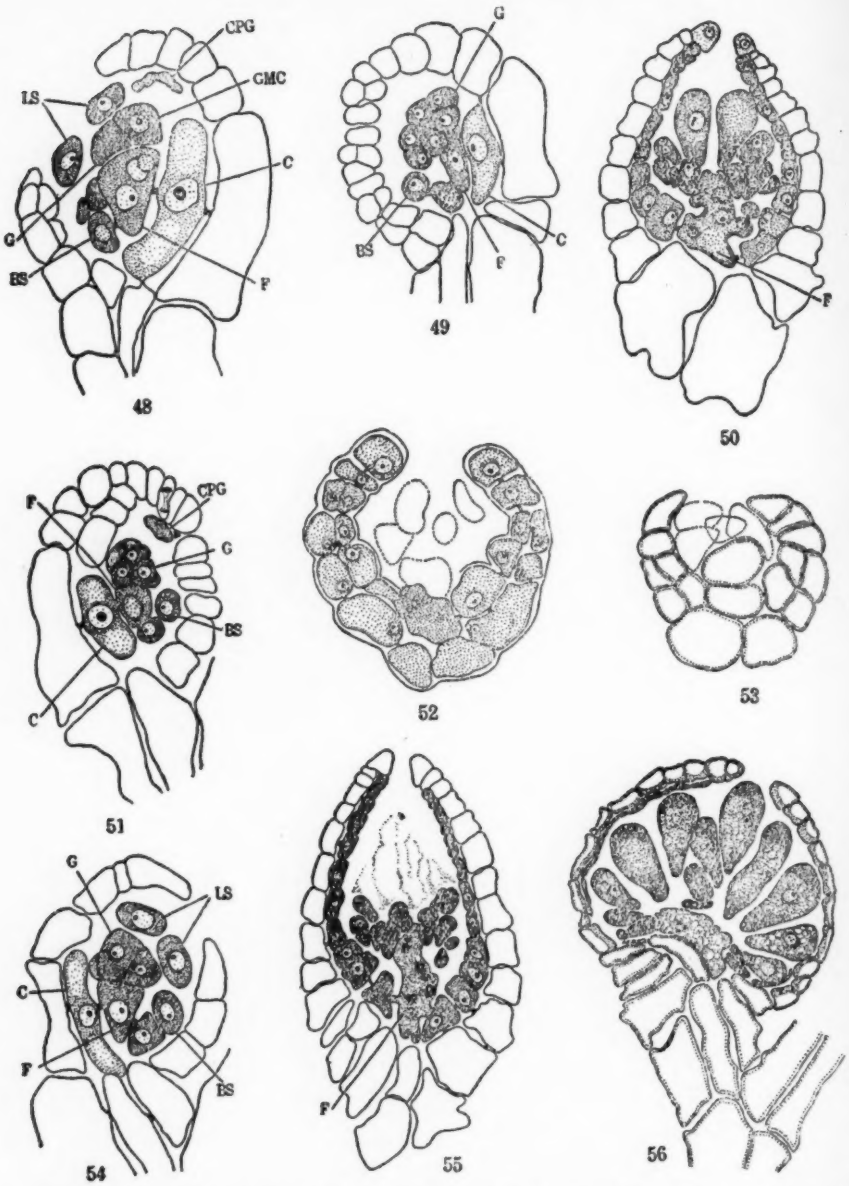


TEXT-FIGS. 34–47. *Polysiphonia platycarpa* Boergs.—Fig. 34. An early stage in the formation of the cystocarpic wall. Fig. 35. A later stage of the same. Fig. 36. Young procarpic segment showing the lateral pericentral cells (L) which give rise to the cystocarp wall (*cf.* Fig. 37). Fig. 37. Schematic representation of a transverse section of a fertile segment, showing the lateral pericentral cells (L) which give rise to the cystocarp wall. Figs. 38–42. Schematic representations of the development of the three-celled carpogonial branch. Fig. 43. Schematic representation of the four-celled carpogonial branch as known in other species of *Polysiphonia*. Figs. 44, 45. Schematic representation of the procarp just before fertilization. Figs. 46, 47. Schematic representation of the procarp just after fertilization. *AU*, auxiliary cell; *BC*, bearing cell; *BS*, basal sterile cells; *C*, central cell; *CBR*, carpogonial branch; *CI*, carpogonial branch initial; *L*, lateral pericentral cells which give rise to cystocarp wall; *LS*, lateral sterile cells; *PC*, fertile pericentral cell. (Figs. 34, 36 × 600; Fig. 35 × 535).

between the carpogonium and the auxiliary cell (Text-Fig. 23). At this stage the fusion nucleus is usually at metaphase and the spindle lies within the carpogonium. The fusion nucleus then enters into anaphase. At this stage one of the daughter groups of chromosomes is seen in the canal-like communication between the carpogonium and the auxiliary cell, as if about to pass into the auxiliary cell, while the other group still remains inside the carpogonium (Text-Fig. 27). This would appear to lend support to Kylin's suggestion that only one group of daughter chromosomes probably passes into the auxiliary cell, while the other remains inside the carpogonium and degenerates. The carpogonium is then cut off by a wall and soon begins to degenerate along with the cells of the carpogonial branch. By this time the protoplasmic connections between the auxiliary cell and the bearing cell and also those between the sterile cells and the bearing cell enlarge so that there is more or less an open communication between all these cells. Also it was observed that, though the sterile cells showed no active cell-division or increase in number, still the nuclei of the sterile cells showed division stages. Some of these nuclei were even seen to migrate through open communications into the bearing cell.

Kylin (1914; 1930, p. 83) has stated that the function of these sterile cells is still not clear. He suggests that they probably prevent the cystocarp wall from being developed too narrowly, and in addition also probably serve as a store-house of reserve food for nourishing the developing gonimoblast. The behaviour of the sterile cells in the present alga would appear to lend support to this suggestion, since we find that the contents of the sterile cells are richer and show nuclear division without the cells themselves increasing in number. Moreover, the slender protoplasmic connections between the bearing cell and the auxiliary cell, as also those between the sterile cells and the bearing cell, become very much broadened so as to leave an open communication between these cells. Further, cytomyxis also was observed between the sterile cells and the bearing cell. All these points would appear to suggest that the sterile cells are probably nutritive in function and nourish the developing gonimoblast.

The fusion nucleus that has entered the auxiliary cell now divides and the auxiliary cell then cuts off the gonimoblast-mother-cell towards its outer side. The gonimoblast-mother-cell cuts off a number of gonimoblast-cells (Text-Figs. 32, 48, 51, 54). These gonimoblast-cells in their turn produce carpospores which are terminal in position and are produced at the end of the gonimoblast-cells. The further development of the gonimoblast-cells takes place laterally. Each of the newly formed gonimoblast-cell produces a



TEXT-FIGS. 48–56. *Polysiphonia platycarpa* Boergs.—Figs. 48, 49, 51 and 54. Stages in the formation of the gonimoblast. Fig. 50. Young cystocarp, showing the two-layered cystocarp

wall, and the dense protoplasmic contents of the cells of the inner layer. Figs. 52, 53. Development of the two-layered cystocarp wall. Fig. 55. Young cystocarp; note the densely protoplasmic cells of the inner layer in open communication with the fusion cell (F). Fig. 56. Ripe cystocarp with fully formed carpospores; note the wall is one-layered, with the remnants of the degenerating inner layer still sticking to it here and there. *BS*, basal sterile cells; *C*, central cell; *CPG*, carpogonium; *F*, fusion cell; *G*, gonimoblast; *GMC*, gonimoblast-mother-cell; *LS*, lateral sterile cells. (Figs. 48, 49, 51-54 × 535; Figs. 50, 55 × 375; Fig. 56 × 165).

terminal carpospore and then divides laterally to form more gonimoblast-cells. This process is repeated until we have a large number of gonimoblast-cells in the cystocarp. Due to the terminal position of the carpospores, the further growth of the gonimoblast is altered from a monopodial type of growth before carpospore formation (Text-Fig. 49) to a sympodial one after the carpospore formation (Text-Fig. 50). The carpospores when fully ripe are ovoid in shape, have very dense protoplasmic contents and are uninucleate (Text-Figs. 50, 56). The carpospores finally break away from the gonimoblast and escape outside through the opening of the cystocarp. Along with the development of the gonimoblast and the formation of carpospores by it, extensive fusions take place, so that we find in the old cystocarp a very large and irregular multinucleate fusion cell (Text-Figs. 50, 55 *F*) which is the result of the fusion of the central cell, the bearing cell, the auxiliary cell, the sterile cells, the gonimoblast-mother-cell and also a large number of gonimoblast-cells themselves. This fusion cell contains a large number of nuclei some of which are presumably haploid and some diploid, the diploid ones being the products of the original fusion nucleus and the haploid ones being derived from the nuclei of the central, the bearing and the sterile cells.

Development of the cystocarp wall

The cystocarp wall takes its origin mainly from the two lateral pericentral cells of the fertile segments, *i.e.*, the third and the fourth pericentral cells (Text-Fig. 37 *L*). In the very young procarp these two cells are prominent because of their large size and large nuclei (Text-Fig. 36). Each of these two pericentral cells begins to divide about the time when the carpogonial branch initial is cut off from the bearing cell. By a series of further divisions, they develop into two protective covering walls like the two shells of an oyster, one on either side of the procarp. At the upper portion there is a space between the two pieces through which the trichogyne projects outside. These two pieces, which together constitute the young cystocarp wall, are one layer of cells in thickness up to the time of fertilization. After fertilization, each cell of the cystocarp wall cuts off a cell to the outside by periclinal division, so that the cystocarp wall which is one layered at first becomes finally two layered (Text-Figs. 52, 53), the outer layer being

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derived from the inner. The corresponding cells of the two layers are connected by primary protoplasmic connections. The cells of the outer layer later on get connected laterally by secondary protoplasmic connections. The cells of the outer layer are poor in contents and are cœnocytic, while those of the inner layer are very rich in contents and uninucleate. The nuclei of the cells of this inner layer are slightly larger than those of the outer. Such of the cells of the inner layer as are in close proximity to the fusion cell enter into open communication with it (Text-Fig. 55), sometimes even fusing with it. Their nuclei also sometimes migrate into the fusion cell.

With the further growth of the cystocarp, an interesting change takes place in the two-layered cystocarp wall. As carpospores are being formed in the cystocarp, the cells of this inner layer become gradually shrivelled up and finally disappear completely. In the oldest cystocarps, the wall consists of only one layer of cells, the original outer layer. Remnants of the inner layer may often be seen sticking to some portions of the outer layer (Text-Fig. 56).

The dense protoplasmic contents of the cells of the inner layer, the establishment of open communication between the cells of this layer and the fusion-cell and the gradual shrivelling up and disintegration of this layer synchronizing with the increased production of the carpospores, all very strongly suggest a nutritive function for the inner layer.

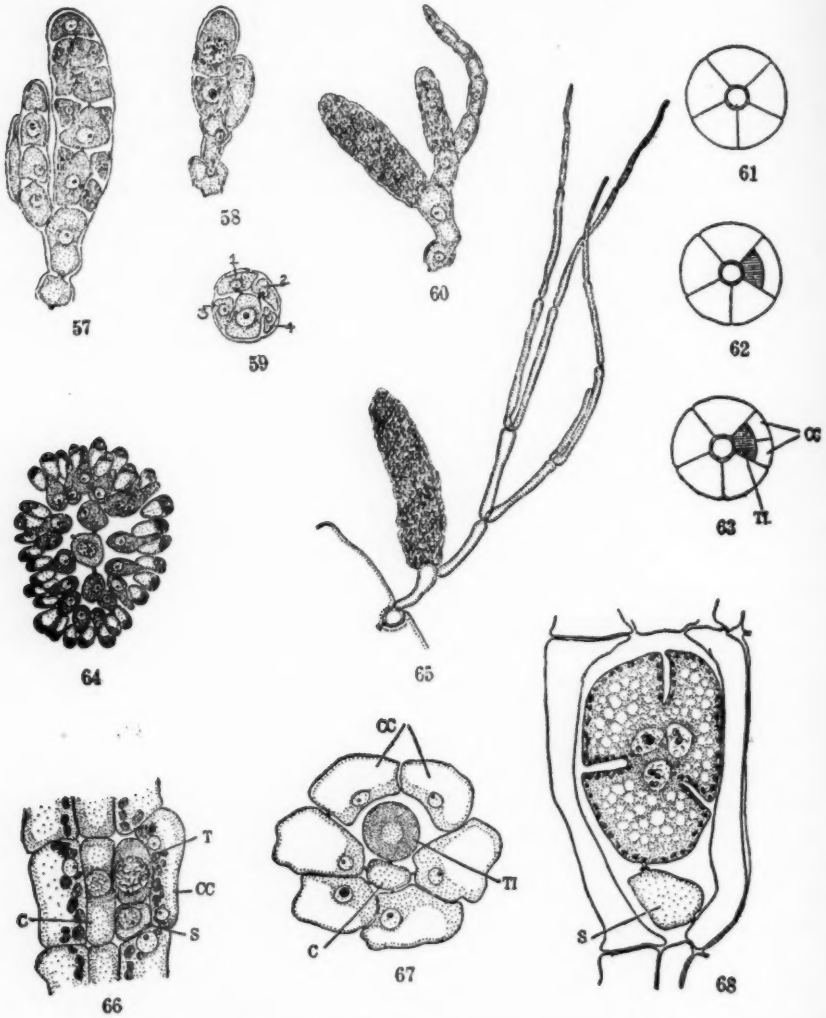
Development of the antheridial clusters

The antheridia are seen as translucent clusters near the apical portions of the male filaments. Each antheridial cluster is borne on a trichoblast on the top of the second cell from the base (Text-Fig. 65). Usually only a single cluster is borne on a trichoblast, but occasionally two antheridial clusters are seen on it (Text-Fig. 60). In such cases the second antheridial cluster is borne on the top of the third cell from the base of the trichoblast. The entire antheridial cluster is clothed by a gelatinous investment, the " cuticle ".

The second segment of the trichoblast which bears the antheridial cluster is somewhat elongated and bears a much branched sterile hair in addition to the antheridial cluster. The antheridial cluster is borne on that side of the trichoblast which is nearest to the main axis. It starts as a single row of cells (Text-Fig. 58). As soon as this row of cells is about 4 or 5 cells long, five pericentral cells are cut off in each segment and the structure soon becomes polysiphonous (Text-Fig. 57). The sequence of events in the formation of the pericentral cells is more or less the same as that described for the

fertile procarpic segment and follows a left-handed spiral scheme (Text-Fig. 59). As a consequence of these divisions, the antheridial cluster is differentiated into a central axis of elongated cells and an outer layer of numerous small pericentral cells. From each pericentral cell are cut off several primary antheridial-mother-cells. From each primary antheridial-mother-cell a number of secondary antheridial-mother-cells are cut off. Thus each pericentral cell bears a number of antheridial-mother-cells which are connected with one another and with the pericentral cell by protoplasmic connections (Text-Fig. 64). This structure is difficult to make out in the young antheridial cluster since the pericentral cells and the antheridial-mother-cells are very closely packed, but in the older clusters the cells become larger and are placed slightly more apart. The structural details of the antheridial cluster of the present form fully agree with those of *Polysiphonia violacea* and *Polysiphonia fastigiata* as recorded by Grubb (1925).

The cytoplasm of the antheridial-mother-cell has a fine granular appearance and is devoid of plastids. The resting nucleus of the antheridial-mother-cell has a nucleolus and a very fine reticulam (Text-Fig. 71). It soon begins to divide. Just about this stage, the antheridial-mother-cell forms a beak-like protuberance at its upper end. At late prophase of the division of the antheridial-mother-cell nucleus, the chromosomes are seen distributed throughout the nuclear cavity as small rods (Text-Fig. 72). The exact number of these chromosomes could not be counted but they appeared to be about 26. During metaphase the chromosomes are arranged in an equatorial plate and a definite spindle could be observed. This spindle is intranuclear (Text-Fig. 69). The nuclear membrane disappears at about late metaphase. The two sets of daughter chromosomes separate from each other at anaphase and in some cases a vacuole is seen between the two daughter chromosome groups as recorded by Yamanouchi (1906 A) in *Polysiphonia violacea.* The spindle is not exactly in the longitudinal axis of the antheridial-mother-cell, but a little oblique to it, so that one of the daughter chromosome groups enters into the beak-like protuberance of the antheridial-mother-cell at late anaphase (Text-Fig. 79). After telophase two daughter-nuclei are formed, one remaining in the broad lower portion and the other in the beak-like protuberance at the top (Text-Figs. 74, 83). This beak-like protuberance with the single nucleus is then abstricted as a young antheridium which remains connected with the parent cell by a delicate protoplasmic strand (Text-Fig. 71). The young antheridium soon increases in size and becomes more or less spherical. In this manner, four or sometimes even five antheridia are successively formed from a single antheridial-mother-cell (Text-Figs. 83-85).



TEXT-FIGS. 57-68. *Polysiphonia platycarpa* Boergs.—Fig. 57. Young antheridial cluster showing pericentral cell formation. Fig. 58. Very young and still monosiphonic antheridial cluster. Fig. 59. Section of a young antheridial cluster, showing the order of pericentral cell formation; nos. 1, 2, 3 and 4 indicate the first, the second, the third and the fourth pericentral cells, respectively. Fig. 60. An unusual case in which two antheridial clusters are borne on a single trichoblast. Figs. 61-63. Schematic representation to show the division of the fertile pericentral cell to form the cover cells (*CC*) and the tetrasporangium initial (*TI*). Fig. 64. Section of a fully developed antheridial cluster, showing the central cell, pericentral cells and the

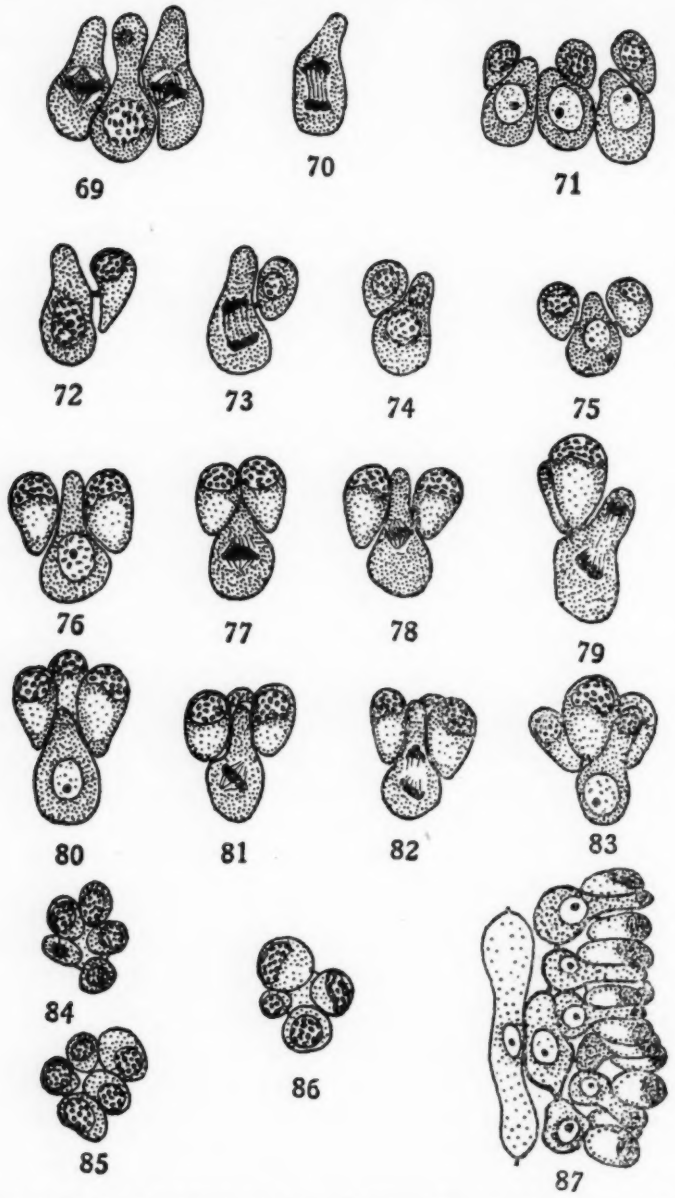
antheridial mother cells. Fig. 65. Antheridial cluster on a trichoblast. Fig. 66. Part of a longitudinal section of a tetrasporangial segment, showing stalk-cell (*S*), tetrasporangium (*T*) and cover-cell (*CC*). Fig. 67. Transverse section of a fertile segment showing the central cell (*C*), tetrasporangium initial (*TI*) and cover-cells (*CC*). Fig. 68. Mature tetrasporangium with the cleavage furrows fairly well advanced, and the stalk-cell (*S*). *C*, central cell ; *CC*, cover cells ; *S*, stalk-cell ; *T*, tetrasporangium ; *TI*, tetrasporangium initial. (Figs. 57–59, 64, 66, 67 × 750 ; Fig. 60 × 375 ; Fg. 65 × 165 ; Fig. 68 × 465).

The formation of the antheridia takes place in a very regular manner and the succession of the antheridia could be clearly followed when seen in surface view (Text-Figs. 84-86). The second antheridium is formed directly opposite to the first and the third and the fourth are formed between the first two (Text-Fig. 86). Thus space is allowed for the first formed antheridia to enlarge and ripen before the other two have attained their full development. This observation is in agreement with that of Grubb (1925) in *Polysiphonia violacea* and *Polysiphonia fastigiata*. The spermatia, when liberated, are sub-spherical or ovoid and have a delicate wall. The nucleus of the spermatium at the time of its liberation is in late prophase and shows a number of large darkly staining granules. The number of these granules could not be counted exactly but appeared to be about 26.

Development of tetrasporangia

The tetrasporangia are formed on distinct tetrasporic plants and are borne on the younger portions of the filaments. Their position in the successive segments closely follows the general left-handed spiral scheme. The segments that form tetrasporangia show five pericentral cells (Text-Fig. 61), while the sterile segments show only four pericentral cells. The fertile pericentral cell divides into two by a periclinal wall (Text-Fig. 62). The outer of these two cells divides by an anticlinal wall into two cells, the cover-cells (Text-Figs. 63, 67 *CC*). The inner cell which is the tetrasporangium initial (Text-Figs. 63, 67 *TI*) then divides transversely in a plane at right angles to the longitudinal axis of the filament, into an upper larger and a lower smaller cell (Text-Fig. 66). Of these the upper cell becomes the tetrasporangium (*T*) while the lower forms the stalk-cell of the tetrasporangium (*S*).

The tetrasporangium soon increases very much in size. The nucleus also becomes larger and shows a faintly staining reticulum (Text-Fig. 94). The cytoplasm is dense and homogeneous and without any conspicuous vacuole. In the slightly older tetrasporangium, the nucleus shows long slender threads distributed in the nuclear cavity (Text-Figs. 66, 97). At a slightly later stage (Text-Fig. 96) there can be seen a slight indication of pairing of the threads here and there (zygotene). A little later (Text-Fig. 95) the threads become slightly thicker and are massed together more or less

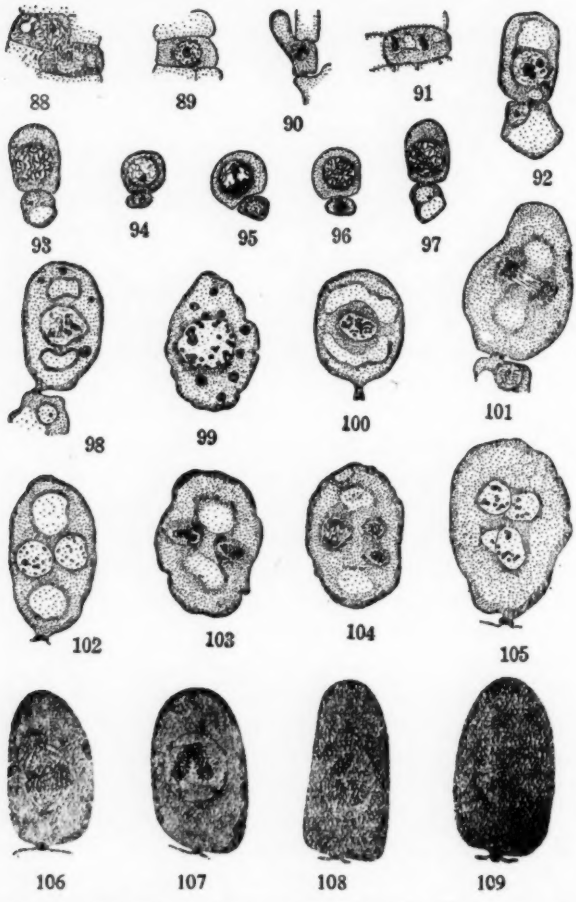


TEXT-FIGS. 69–87. *Polysiphonia platycarpa* Boergs.—Figs. 69, 70. Stages in the division of the antheridial mother-cell preceding the formation of the first antheridium. Fig. 71. Antheridial mother-cells each with one antheridium formed already. Figs. 72–75. Stages in the

formation of the second antheridium. Figs. 76–80. Stages in the formation of the third antheridium. Figs. 81–83. Stages in the formation of the fourth antheridium. Figs. 84, 85. Surface view of antheridial mother-cell, showing five fully formed antheridia. Fig. 86. Surface view of antheridial mother-cell showing four fully formed antheridia ; note the two younger antheridia are formed between the two older ones. Fig. 87. Longitudinal section of an antheridial cluster, showing the central cell, a pericentral cell and a number of antheridial mother-cells with ripe antheridia. (All × 2250).

at one side of the nuclear cavity (synizesis). After synizesis, the threads become redistributed throughout the nuclear cavity and at this stage (Text-Fig. 93) the paired nature is clearly seen (pachytene). After pachytene, a very peculiar behaviour is seen in the nucleus. The nucleus passes into an apparent resting stage (Text-Fig. 92). At this stage the nucleus shows only a very faintly stained reticulum and the nucleolus often shows an irregular shape and a tendency to fragment. The nucleus remains in this apparent resting condition until the tetrasporangium enlarges considerably. With the increase in size of the tetrasporangium, the nucleus also enlarges, though still remaining in the apparent resting condition. At about this stage certain deeply staining bodies make their appearance in the cytoplasm of the tetrasporangium (Text-Fig. 98). These darkly staining bodies disappear about metaphase. Their exact nature is not very clear. The intervention of an apparent resting condition between pachytene and diakenesis was first recorded by Westbrook (1928, p. 160) in *Rhodymenia palmata* and later on by her in a number of other members of the Florideæ (Westbrook, 1928; 1935). This resting stage was compared by her with the " diffuse " or " confused " stage of the nucleus seen during meiosis in developing animal oocytes, where in the normal meiotic prophase, the chromosomes become invisible due to the deconcentration of stainable substances, the chromatin becoming thereby difficult to detect. Westbrook comes to the conclusion that this condition of the nucleus is conneted with a synthetic phase and is correlated with the attainment of a stage in the tetrasporangium in which a certain size is reached and reserve food material begins to accumulate. The appearance of darkly staining bodies in the cytoplasm of the tetrasporangium during the resting stage and their disappearance before metaphase, appear to suggest that they probably represent the reserve food material referred to by Westbrook.

Diakinesis is seen only after the tetrasporangium attains a large size. At this stage, the chromosomes are seen to be excessively shortened and have the appearance of very short rods distributed in pairs in the nuclear cavity near its periphery (Text-Fig. 99). The nucleolus could be seen as a less deeply stained body than the chromosomes. At times only a single nucleolus is present (Text-Fig. 100), but sometimes more than one nucleolus



TEXT-FIGS. 88–109. *Polysiphonia platycarpa* Boergs.—Figs. 88–91. Somatic mitosis in the tetrasporic plant. Fig. 92. Tetrasporangium with the nucleus in an apparent resting condition between pachytene and diakinesis. Figs. 93–97. Prophase stages of the reduction division. Fig. 93. Pachytene; note the paired nature of the threads. Fig. 94. Young tetrasporangium, showing the resting nucleus. Fig. 95. Synizesis. Fig. 96. Zygotene. Fig. 97. Leptotene. Fig. 98. Tetrasporangium with the nucleus in the apparent resting condition, at a later stage than in Fig. 92 ; note the darkly staining bodies in the cytoplasm. Fig. 99. Diakinesis ; only 20 pairs of chromosomes shown in the figure. Fig. 100. Late diakinesis, showing all the 26 pairs of chromosomes. Fig. 101. Late anaphase of the first division ; note the axis of the spindle is nearly at right angles to the longitudinal axis of the tetrasporangium. Fig. 102. Binucleate tetrasporangium. Fig. 103. Metaphase of the second division. Fig. 104. Anaphase of the second division ; note the axes of the two spindles are not perpendicular to each other. Fig. 105. Four-nucleate tetrasporangium. Text-Figs. 106–109. Stages in reduction division in *Polysiphonia violacea* (After Yamanouchi). (Figs. 88–105 × 750).

could be seen (Text-Fig. 99). At this stage 26 pairs of chromosomes could be easily counted. In the two other species of *Polysiphonia* previously investigated cytologically, *viz.*, *P. violacea* (Yamanouchi, 1906 A) and *P. nigrescens* (Kylin, 1923), the number of chromosomes reported was $X = 20$.

The only other stage of the heterotypic division observed was late anaphase. The axis of the spindle was not parallel to the longitudinal axis of the tetrasporangium as reported for *Polysiphonia violacea* (Yamanouchi, 1906 A) and *Rhodomela virgata* (Kylin, 1914), but was very nearly at right angles to it (Text-Fig. 101). Finally, two daughter nuclei are organized (Text-Fig. 102; Plate II, Fig. 5). The two newly formed daughter-nuclei rest for a time before entering on the homeotypic division.

During the second division, the two nuclei divide simultaneously. The axes of the two spindles are usually at right angles to each other (Text-Fig. 103; Plate II, Fig. 2), though this is not always the case (Text-Fig. 104). The nuclear membrane and the nucleolus seem to disappear at late metaphase. Anaphase and telophase soon follow and four daughter-nuclei are organized in the tetrasporangium (Text-Fig. 105). These four nuclei are seen to occupy more or less a central position in the tetrasporangium.

After the formation of the four daughter-nuclei, cleavage furrows start from the periphery of the tetrasporangium and progress inwards and finally four tetraspores are formed. The ripe tetraspore is naked and densely protoplasmic. It is uninucleate and numerous discoid plastids are seen scattered near the periphery. In a few cases the tetraspores appeared as if escaping outside by squeezing themselves out through amœboid movement between the two cover cells of the fertile segment (Pl. VII, Fig. 4).

Yamanouchi (1906 A, p. 423) has described the reduction division in *Polysiphonia violacea* as follows:

"The spindle of the first mitosis is of very short duration. As soon as the chromosomes at the equatorial plate separate into two groups, the two spindles of the second mitosis suddenly appear and the first spindle can no longer be recognized (Fig. 157). It is possible that the two poles of the first spindle may move along the membrane and become poles of the second spindle, but I have no evidence to support this suggestion. Whatever the origin of the poles of the second mitosis, they are placed in an entirely different position from the poles of the first. The second mitosis in the tetraspore mother cell, therefore, follows so shortly after the first that there is no period between for the organization of two resting nuclei.

"The rapidity with which the second mitosis follows the first may prevent the organization of centrosphere-like structures at the poles. The axis of the two spindles of the second mitosis lie perpendicular to each other (Figs. 157, 158), and the two nuclear divisions take place simultaneously. At anaphase four groups of chromosomes, 20 in number, pass to the poles, still included in the membrane of the original tetraspore mother cell, which persists from the beginning (Fig. 159)."*

The reduction division in *Polysiphonia violacea* as recorded by Yamanouchi shows some very extraordinary features. Firstly, the first division proceeds as far as metaphase and the two groups of daughter chromosomes are just beginning to separate, when suddenly the second division sets in. And the second division starts at metaphase. In other words, both the divisions of the reduction division are incomplete. In the first division, the later stages (*i.e.*, anaphase and telophase) are absent, and, in the second division, the earlier stages (*i.e.*, prophase stages) are absent. Secondly, there is no interkinesis. Two resting nuclei are not organized before the commencement of the second division. Thirdly, we have the extraordinary condition of the two spindles of the second division lying enclosed within the old nuclear membrane of the original tetrasporangium nucleus.

In the present species (*Polysiphonia platycarpa*) none of these extraordinary features is seen. The reduction division is quite normal throughout. The nuclear membrane of the tetrasporangium nucleus disappears before the late anaphase of the first division. The first division is completed and two resting nuclei are organised each with its own nuclear membrane. The second division is again quite normal. Two separate spindles are formed. The nuclear membrane of each of these two dividing nuclei disappears at about metaphase and the two spindles lie apart in the cytoplasm of the tetrasporangium. After the telophase of the second division, four separate nuclei, each with its own nuclear membrane, are formed. Thus the reduction in the present form is quite normal and not at all like that of *Polysiphonia violacea* as recorded by Yamanouchi, but more like that of *Rhodomela virgata* as described by Kylin (1914).

Discussion

The carpogonial branch

In all the members of the Rhodomelaceæ so far investigated, in fact, in the entire order Ceramiales, only four-celled carpogonial branches have

* Yamanouchi's Figs. 156, 157, 158 and 159 are reproduced in the present paper as Text-Figs. 106, 107, 108 and 109, respectively.

been recorded* (*cf.*, Kylin, 1930, pp. 61-63; 1937, p. 261). It is very interesting, therefore, to find that the carpogonial branch is three-celled in the present alga.

The origin and development of the cystocarp wall

Two views have been expressed regarding the origin of the different layers of the cystocarp wall in the Rhodomelaceæ. Phillips (1895, p. 301) states that the cystocarp wall is composed of only one layer of cells and that it is lined on the inside by a set of filaments—the " paranematal filaments ". He states that these paranematal filaments arise directly from the central cell after fertilization and thinks that they probably serve the purpose of supporting the cystocarp wall. This opinion has been restated by Yamanouchi (1906 A, p. 420) and later on by Schussnig and Jahoda (1927, p. 236). The two latter authors state that the inner layer is a post-fertilization development arising out of the central cell and hence does not belong to the cystocarp wall proper. They consider the cystocarp wall as consisting of only one layer of cells and to have been derived by the concrescent growth of trichoblasts which have grown together congenitally.

Falkenberg (1901, p. 105) on the other hand expresses the view that the cystocarp wall is formed out of the outgrowing polysiphonous lateral branches which have grown together concrescently and congenitally. He considers the inner layer as the central cells of these branches and the outer layer as the pericentral cells. If there are further outer layers as in some other members of the Rhodomelaceæ, he considers them to be the cortical layers of the branches. In support of this view, he quotes instances of cystocarpic proliferations where the cystocarp wall grows out into polysiphonous branches (Falkenberg, 1901, p. 105, Fig. 14). Falkenberg's opinion has been confirmed by Connolly (1911, p. 135), Kylin (1914, p. 53; 1923, p. 121; 1930, p. 81; 1937, p. 291) and Rosenberg (1933, p. 27). According to these authors, the first formed layer is the inner layer and the outer layer is cut off from this inner layer, so that the inner layer is really the older, whereas according to Phillips and others, the outer layer is the older and the inner the younger.

Kylin (1930, pp. 81-83) does not agree with Schussnig and Jahoda's statement that the inner layer of the cystocarp wall arises out of the central

* It may be mentioned here that a three-celled carpogonial branch was recorded by Schussnig and Odle (1927, pp. 237, 243) in *Spermothamnion roseolum*. But Kylin, who investigated this alga again in 1930, showed that the interpretation of Schussnig and Odle was wrong and that the carpogonial branch in *Spermothamnion roseolum* was really four-celled (Kylin, 1923; 1930, p. 62). Drew (1934) found that the carpogonial branch of another species of *Spermothamnion*, (*S. Turneri*), was also four-celled.

cell. He says that the fertile central cell cuts off five pericentral cells around it and remains connected with them right from the beginning and that it is therefore impossible for it to cut off any more pericentral cells.

The writers' observations on the development of the cystocarp wall in the present alga lend full support to the opinion expressed by Falkenberg, Kylin and others. In the present alga the cystocarp wall always commences as a single-layered one which becomes two layered later on by cutting off a second layer to the outside. This outer layer is composed of merely the pericentral cells cut off by the cells of the inner layer.

Since all the cells of the outer layer are derived from cells of the inner layer, the cells of the outer layer at first have protoplasmic connections only with their corresponding sister cells of the inner layer. It is only later on that the cells of the outer layer form protoplasmic connections laterally among themselves. And, when the inner layer gets disorganised and finally withers away, almost all the primary connections of the cells of the outer layer with those of the inner layer get lost and we have then the remarkable condition of having a cystocarp wall which is composed only of the pericentral cells of the concrescently outgrowing polysiphonous lateral branches, held together by their secondarily formed protoplasmic connections.

A few previous workers have noticed the densely protoplasmic nature of the inner layer; and some of them have suggested a nutritive function for this inner layer. But the gradual shrivelling up of the inner layer as more and more carpospores are formed does not appear to have been recorded for any of the members of the Rhodomelaceæ. In the present form the dense protoplasmic contents of the cells of the inner layer, the establishment of open communication between the cells of this layer and the fusion cell and the fact that the gradual shrivelling up and disintegration of this layer synchronizes with the increase in the production of the carpospores lend full support to the suggestion of a nutritive function for this inner layer.

The figures of cystocarps of *Brongniartella byssoides* given by Schussnig and Jahoda (1927, p. 236, Fig. 11 *b*) and of *Polysiphonia nigrescens* given by Phillips (1895, Pl. X, Figs. 7 and 8), show a shrivelled up condition of the inner layer. In these forms also a similar behaviour of the inner layer appears probable. A detailed study of some other members of the Rhodomelaceæ also with reference to this point will prove interesting.

The reduction division

A peculiar and very extraordinary mode of ruduction division was recorded in *Polysiphonia violacea* by Yamanouchi. In this reduction divi-

sion, the two divisions of meiosis are very much abridged and follow each other very rapidly without interkinesis at the end of the first division. Since this is the only species of *Polysiphonia* investigated so far as regards reduction division, Kylin accepted this type of reduction division as a condition obtaining in the genus *Polysiphonia*. He says, in his *Anatomie der Rhodophyceen* (1937, p. 9), "Bei *Polysiphonia* entwickeln sich dagegen die beiden homöotypischen Spindeln innerhalb der noch persistierenden Membran des primären Tetrasporangienkerns." The reduction division in the present species (*Polysiphonia platycarpa*) is quite normal and not at all like that of *Polysiphonia violacea*. Kylin's statement quoted above would therefore have to be modified as applying to *Polysiphonia violacea* only and not to all the species of the genus. It would be interesting to investigate the reduction division in other species of *Polysiphonia* also and find out how many of them conform to the *Polysiphonia violacea* type of reduction division and how many to the normal type of reduction division as seen in the present species, *Polysiphonia platycarpa*.

Summary

Polysiphonia platycarpa Boergs. occurs during certain parts of the year in the estuarine portion of the river Adyar at Madras, and in the backwater at Ennur eleven miles north of Madras.

The structure and development of the thallus was followed in detail.

The details of the development of the procarp before and after fertilization agree generally with those already recorded for the other members of the Rhodomelaceæ. The present alga, however, differs from all the previously investigated members of the family in having a three-celled carpogonial branch instead of the usual four-celled one.

Fusion between the carpogonium and the auxiliary cell is effected by means of a connecting cell which is cut off from the fertilised carpogonium.

A nutritive role is suggested for the lateral and the basal sterile cells.

The cystocarp wall is two-layered at the beginning, the outer layer being derived from the inner. During further development, the cells of the inner layer, which are rich in contents, gradually shrivel up and finally disappear altogether, so that the wall of the mature cystocarp is composed of only a single layer of cells, *viz.*, the original outer layer. The original inner layer is evidently nutritive in function.

The structure and development of the antheridial cluster and of the antheridia are described in detail.

Reduction division was followed in detail in the tetrasporangium. The peculiar mode of reduction division recorded by Yamanouchi in *Polysiphonia violacea*, where the metaphase of the second division follows immediately after early anaphase of the first division, the intervening stages being completely dropped, is not seen in the present alga. The reduction division in the present form is quite normal with a definite interkinesis between the first and the second division.

An apparent resting stage intervenes between pachytene and diakinesis similar to the one recorded by Westbrook in some members of the Florideæ.

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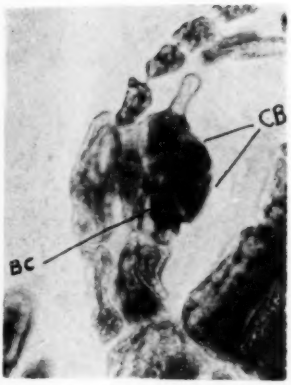

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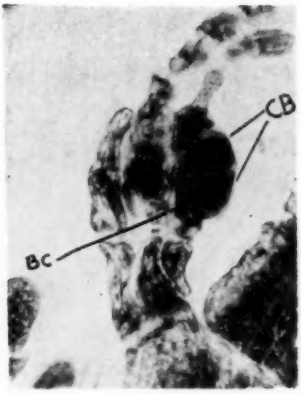

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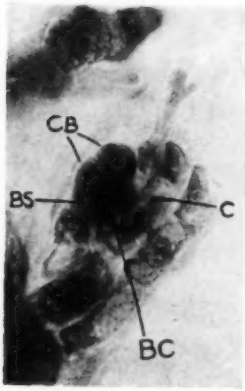

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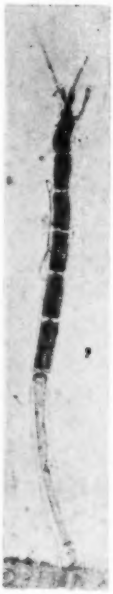

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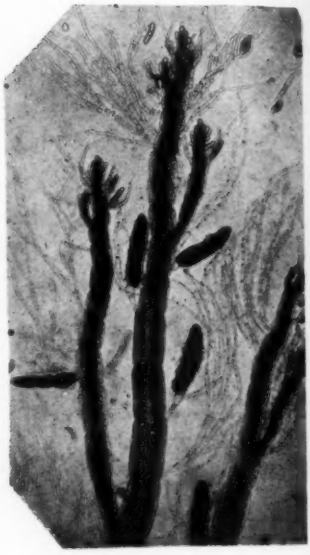

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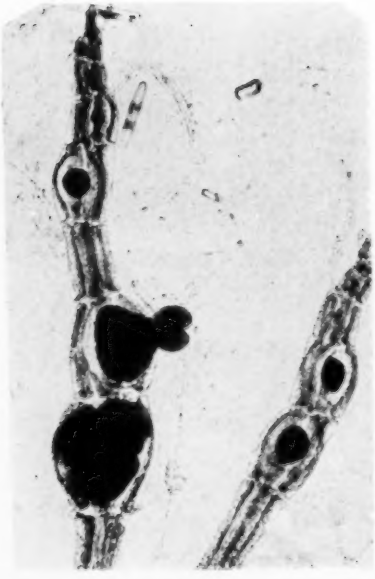

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6

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Explanation of Plates

PLATE VI

Polysiphonia platycarpa Boergs.

Figs. 1, 2. Photomicrographs of a young procarp taken at two different foci, showing the three-celled carpogonial branch (*CB*) and the bearing cell (*BC*). × 950.

Fig. 3. Young procarp, showing central cell (*C*), bearing-cell (*BC*), three-celled carpogonial branch (*CB*) and basal sterile cell (*BS*); note forked trichogyne. × 950.

Fig. 4. Section of young cystocarp showing developing carpospores and the two-layered wall. × 350.

Fig. 5. A sporeling. × 110.

Fig. 6. Part of a female plant with a young and an old cystocarp. × 160.

PLATE VII

Polysiphonia platycarpa Boergs.

Fig. 1. Growing apex of a female plant showing apical cell and a young procarp initial. × 330.

Fig. 2. Section of tetrasporangium showing the metaphase of the second division. × 1,450.

Fig. 3. Apical portion of a male plant showing antheridial clusters. × 75.

Fig. 4. Portions of a tetrasporic plant with tetrasporangia; note the escape of tetraspores in one of the sporangia. × 150.

Fig. 5. Tetrasporangium showing the two-nucleate stage; note the two prominent vacuoles above and below the two nuclei (*cf.* Text-Fig. 102). × 1,140.

Fig. 6. Habit of the alga, growing epiphytically on a leaf of *Halophila ovalis*. About natural size

MARINE PLANKTON FROM WATERS AROUND THE KRUSADAI ISLAND

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I. Introduction

Though our knowledge of the plankton of the Indian seas is not considerable, the contributions of Menon, K. S. (1931), Aiyar, Menon and Menon (1936), Devanesen (1942), Chidambaram and Menon (1945) and Menon, M. A. S. (1945) are noteworthy as they have brought much valuable data on the planktonic organisms in different parts of the Indian coast. The present paper deals with the results of an investigation of the plankton of the sea around the Krusadai Island in the Gulf of Manaar from September 1940 to August 1943, during which period 436 samples collected thrice a week have been studied both qualitatively and quantitatively. Horizontal hauls were made with a simple tow-net from a rowing boat or from the motor launch " Pearl " for ten minutes from the sea ranging from 1 to 12 fathoms in depth, both during day and night.

II. Physical and Hydrographical Features of Waters around Krusadai Island

Krusadai (Fig. 1) is one of a string of coral islands running approximately east and west and parallel to the Mandapam Peninsula, and lies off the Island of Rameswaram in the Gulf of Manaar in Latitude 9° 14′ N. and Longitude 79°·12′ E. It is exposed to both the south-west and north-east monsoons, the latter being of more intensity. The general drift of water in the Gulf is from the south to the north from about the end of April to the end of September, and in the reverse direction during the height of the north east monsoon. The hydrographical conditions of this region are given in the following Table (Table I). Salinity was determined by the Mohr's method of chlorine titration with silver nitrate using potassium chromate as indicator. The readings were corrected with that of ' Standard Sea Water ' obtained from the Laboratoire Hydrographique, Copenhagen; and then converted into salinity by means of Kundsen's (1901) Hydrographical Tables. Specific gravity was determined by means of the common hydrometers, the readings of which were corrected to temperature. The

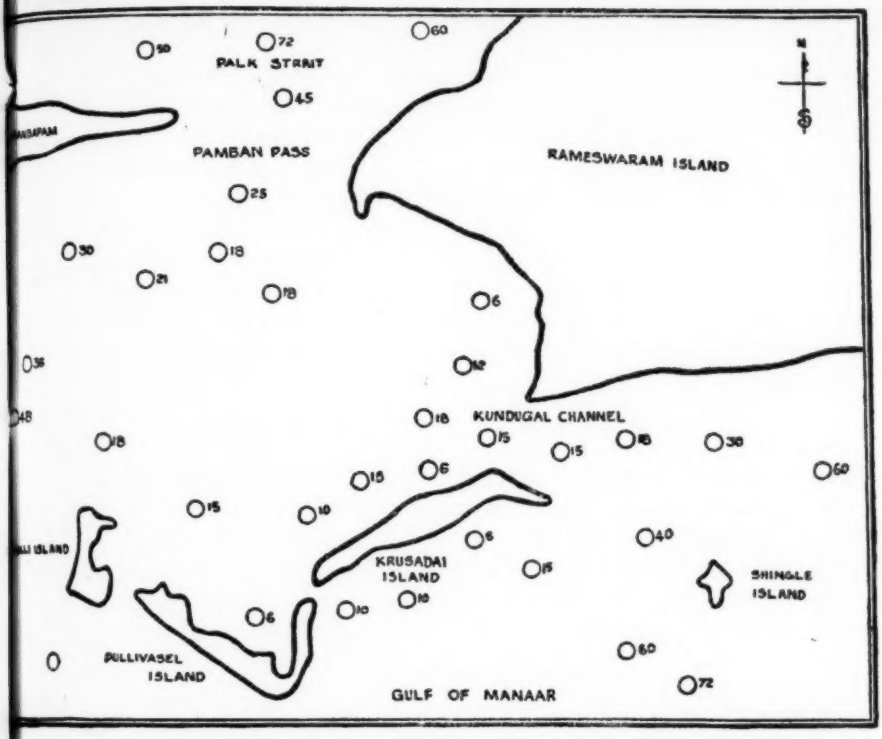

FIG. 1. Krusadai Island and surrounding sea showing the depth of the water in feet at the principal plankton collecting stations. (Scale 1 inch : 15 miles)

TABLE I

Hydrographical conditions in the Kundugal Channel near Krusadai Island, 1942

Month	Salinity o/oo	Surface temperature °C.	pH	Specific gravity	Rainfall in inches
January ..	28·40	25·2	8·4	1·0120	3·21
February ..	32·59	26·7	8·5	1·0220	0·10
March ..	34·26	28·2	8·5	1·0231	0·65
April ..	34·30	29·8	8·5	1·0231	0·65
May ..	34·60	29·5	8·5	1·0233	2·93
June ..	35·12	28·3	8·5	1·0242	0·30
July ..	35·36	28·1	8·6	1·0252	0·10
August ..	35·40	28·3	8·6	1·0252	0·30
September ..	35·70	28·3	8·5	1·0256	0·48
October ..	36·20	28·5	8·5	1·0248	5·00
November ..	30·10	26·9	8·5	1·0200	21·80
December ..	25·88	26·2	8·4	1·0218	3·00

pH was estimated by using 0·5 c.c. of metacresol purple solution to 10 c.c. of the sample and by comparing the colour produced with standard B.D.H. tubes. The hydrographical data given in Table I represent monthly averages of readings taken from surface samples of sea water collected from the Kundugal channel, on every day excepting Sundays. The water was collected for analysis during the mornings between 8 and 9 A.M., at which period the other readings were also taken. As the laboratory and working facilities were meagre, these figures are probably not useful in interpreting the finer chemical changes, but nevertheless give an indication of the course of monthly and seasonal changes especially in conjunction with the rainfall.

III. Seasonal Distribution of the Organisms

The frequency occurrence of the various planktonic organisms collected during the different seasons is tabluated below (Table II). In making the quantitative representation, the terms rare (*r*), few (*f*), common (*c*), plenty (*p*) and swarms (*s*) are applied in increasing order to denote 1–5, 5–20, 20–100, 100–200, and more than 200 of the organisms in 1 c.c. of the plankton. The macroplanktonic organisms were however counted from the entire haul.

IV. General Discussion

The plankton of the waters around Krusadai is rich both in bulk and variety. Most of the important species appear to be cosmopolitan in distribution. The maximal period of phytoplankton is from June to November, and that of zooplankton from October to April. A comparison can be made here with the observations of workers in other parts of India. In Bombay, the period of phytoplankton scarcity is from May till August after which there is an increase till the maximum is reached in January and February (Gonzalves, 1947), and the zooplankton maxima range from January to March (Bal and Pradhan, 1945). In Madras, the maxima of phytoplankton are in April and May and also in September and that of Zooplankton from October tc March (Menon, K. S., 1931). In Trivandrum, the diatom maximum is from April to May, and the zooplankton zenith from December to March (Menon, M. A. S., 1945). Along the coast of Calicut the maximum productivity of phytoplankton and zooplankton takes place from May to October and from November to April respectively (Hornell and Nayudu, 1923). Although it is difficult to make detailed comparisons at this stage, the fact that the zooplankton maxima fall about the same period in the Madras area and the Krusadai area is worthy of note.

TABLE II

Monthly incidence of the organisms of the plankton

Name of organism	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
DIATOMEÆ												
COSCINODISCEÆ												
1. *Paralia sulcata* (Cleve)	r	r	.	.	r	r	r	r	.	.	r	r
2. *Stephanophyxis palmeriana* (Greville) ..	r	.	.	r	r	.	.	r	r	.	.	r
3. *Sceletonema costatum* (Greville) ..	r	r	r	r	.	.	r	r	r	r	r	r
4. *Thalassiosira decipiens* (Grunow) ..	r	r	r	r	r	f	f	r	r	r	r	r
5. *Thalassiosira condensata* (Cleve) ..	r	.	r	r	.	.	.	r	r	r	r	r
6. *Coscinosira polychorda* (Gran) ..	f	f	r	r	.	.	r	r	r	r	f	f
7. *Cyclotella meneghiniana* Kutzing ..	r	.	r	.	.	.	.	r	.	.	r	r
8. *Coscinodiscus excentricus* Ehrenberg ..	r	r	.	r	f	.	r	r	r	.	.	f
9. *Coscinodiscus lineatus* Ehrenberg ..	r	.	.	r	r	.	.	.	r	.	.	r
10. *Coscinodiscus jonesianus* (Greville) ..	c	c	c	f	f	f	c	c	c	c	c	c
11. *Coscinodiscus concinnus* W. Smith ..	.	f	r	.	.	.	r	r	r	.	.	f
12. *Coscinodiscus oculus-iridis* Ehrenberg ..	f	f	f	r	f	.	c	r	f	f	c	c
13. *Coscinodiscus gigas* Ehrenberg ..	c	f	f	f	c	c	p	c	p	p	p	c
14. *Coscinodiscus nobilis* (Grunow) ..	f	f	r	r	.	.	f	f	f	r	.	r
15. *Planktionella sol* (Wallich)	r	r	.	.	r	.	r	.	r	r	r	r
ACTINODISCEÆ												
16. *Actinopthchus undulatus* (Bailey) ..	.	.	r	r	.	.	.	.	r	r	.	.
SOLNIEÆ												
17. *Lauderia annulata* Cleve	r	r	.	.	.	.	.	.	.	r	r	r
18. *Detonula schroderii* (Bergon)	.	r	.	.	r	r	.	r	r	.	r	.
19. *Leptocylindrus danicus* Cleve	r	.	.	.	.	r	.	.	r	r	.	r
20. *Guinardia flaccida* (Castracane) ..	.	r	.	r	r	r	.	.	r	r	r	.
21. *Rhizosolenia stolterfothi* Peragallo ..	r	r	f	r	.	.	r	r	.	r	.	r
22. *Rhizosolenia imbricata* Brightwell ..	r	f	f	r	.	.	r	f	r	f	f	r
23. *Rhizosolenia styliformis* Brightwell ..	s	c	f	f	f	f	c	p	c	c	p	c
24. *Rhizosolenia setigera* Brightwell ..	r	r	c	f	f	f	f	f	c	p	r	r
25. *Rhizolosenia alata* Brightwell	c	f	c	f	f	p	s	p	c	c	p	c
26. *Rhizosolenia calcaravis* Schultze ..	r	r	.	.	r	f	c	c	f	c	.	.
27. *Rhizosolenia clevi* (Ostenfeld)	r	.	r	r	r	.	r	r	r	.	r	.
CHÆTOCEREÆ												
28. *Bacteriastrum varians* Lauder	r	r	r	r	r	r	r	r	r	r	r	r
29. *Chætoceras coarctatus* Lauder ..	c	f	f	c	p	p	c	c	p	p	c	c
30. *Chætoceras peruvianus* Brightwell ..	c	f	c	c	p	p	c	f	c	p	c	c
31. *Chætoceras lorenzianus* Grunow ..	f	f	c	f	f	p	c	c	p	p	p	f
32. *Chætoceras constrictum* Gran	c	c	f	c	c	c	p	p	p	p	c	c
33. *Chætoceras debile* Cleve	f	c	f	f	f	c	p	c	f	f	c	c
34. *Chætoceras didymus* Ehrenberg ..	r	f	f	r	.	.	r	r	.	r	r	r
35. *Chætoceras affinis* Lauder	r	.	f	r	.	.	r	f	r	r	f	r

TABLE II—(*Contd.*)

Name of organism	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
BIDDULPHIEÆ												
36. *Eucampia zodiacus* Ehrenberg ..	r	.	f	r	.	.	r	f	r	r	f	r
37. *Climacodium frauenfeldianum* Grunow ..	r	.	.	.	r	r	.	.	.	.	r	r
38. *Streptotheca thamensis* Shrubs ..	.	r	.	.	r	r	.	.	.	r	.	.
39. *Bellarochea malleus* (Brightwell) ..	.	.	r	.	.	r	r	.	.	.	r	.
40. *Ditylum Brightwellii* (West)	.	r	.	r	.	.	.	r	r	.	.	.
41. *Ditylum sol* Grunow	r	r	r	r	r	r	r	r	r	r	r	r
42. *Triceratium reticulum* Ehrenberg ..	.	r	.	r	r	.	.	.	r	.	r	.
43. *Biddulphia sinensis* Greville ..	r	r	r	r	r	r	r	r	f	c	c	r
44. *Biddulphia mobiliensis* Bailey	r	r	.	f	f	r	r	.	r	r	r	.
HEMIAULINEÆ												
45. *Hemialus sinensis* Greville	r	.	r	r	r	.	.	r	r	.	.	.
EUODIEÆ												
46. *Hemidiscus Hardmannianus* (Grecille) ..	f	c	r	r	c	r	.	.	f	f	c	f
FRAGILARIOIDEÆ												
47. *Rhabdonema mirificum* W. Smith ..	.	r	r	.	.	.	.	r	.	.	.	.
48. *Gramatophora undulata* Ehrenberg ..	.	r	.	.	.	.	r	r	.	.	r	.
49. *Licomorpha lyngbyei* (Kutzing) ..	r	.	r	r	.	.	.	.	.	.	.	r
50. *Fragilaria oceanica* Cleve	c	f	r	f	c	c	f	r	r	f	c	c
51. *Thalassionema nitzschioides* Grunow ..	r	r	r	r	r	r	r	r	r	r	r	r
52. *Thalassiothrix longissima* Cleve ..	c	f	c	f	f	p	p	c	c	p	p	c
53. *Thalassiothrix Frauenfeldii* Grunow ..	c	c	p	p	f	c	p	p	p	c	c	c
54. *Asterionella japonica* Cleve	f	r	r	s	r	f	f	f	r	r	f	r
NAVICULOIDEÆ												
55. *Mastogloia exilis* Hustedt	.	.	r	r	.	.	.	.	.	r	.	r
56. *Gyrosigma balticum* (Ehrenberg) ..	.	r	r	.	.	.	r	.	r	r	r	.
57. *Pleurosigma elongatum* W. Smith ..	r	r	f	f	r	r	f	r	f	r	f	f
58. *Diploneis Weissflogii* (A. Schmidt) ..	.	.	.	r	.	.	.	r	r	.	.	r
59. *Navicula long* (Gregory)	r	r	r	r	r	r	r	r	r	r	r	r
60. *Amphiprora gigantea* Grunow ..	r	r	.	.	.	r	r	.	.	r	.	.
NITZSCHIACEÆ												
61. *Bacillaria paradoxa* Gmelin	f	r	r	.	r	f	.	r	f	f	r	f
62. *Nitzschia sigma* (Kutzing)	f	f	c	c	r	r	f	r	f	c	f	f
63. *Nitzschia closterium* (Ehrenberg) ..	f	f	r	f	r	r	f	r	r	f	r	f
64. *Nitzschia longissima* (Brebisson) ..	f	f	f	f	.	r	f	r	f	f	f	f
65. *Nitzschia seriata* (Cleve)	f	f	r	r	.	.	r	.	r	f	f	r
MYXOPHYCEÆ												
66. *Oscillatoria priceps* Vauch	.	.	.	.	.	r	.	.	r	.	r	r
67. *Lyngbya æsturaii* (Martens)	.	.	.	.	.	.	r	.	.	.	r	r
CHLOROPHYCEÆ												
68. *Enteromorpha intestinalis* (L.)	.	.	r	r	.	.	.	r	.	r	r	r
69. *Enteromorpha compressa* (L.)	.	.	.	r	.	.	.	r	.	.	r	r
70. *Cladophora glomerata* (L.)	r	r	.	r	.	r	r	.	.	r	r	.
71. *Codium tenue* Kutzing	r	.	.	r	r	.	.	.	.	.	r	r

TABLE II—(*Contd.*)

Name of organism	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
RHODOPHYCEÆ												
72. *Gracilaria lichenoides* (L.)	*r*	*r*	.	.	*r*	*r*	.	.	.	*r*	*r*	*r*
73. *Gracilaria confervoides* (L.)	*r*	.	.	.	.	*r*	.	.	.	.	*r*	*r*
74. *Polysiphonia sertularioides* (Grat) ..	*r*	.	.	*r*	.	.	*r*	.	.	.	*r*	*r*
75. *Polysiphonia subtilissima* Mont. ..	*r*	.	*r*	*r*	.	.	.	.	.	*r*	*r*	*r*
76. *Ceramium elegans* Ducl.	.	*r*	*r*	*r*	.	.	*r*	.	*r*	*r*	*r*	.
SCHIZOPHYCEÆ												
77. *Trichodesmium erythræm* Ehrenberg ..	*p*	*c*	*c*	*s*	*s*	*f*	*s*	*c*	*p*	*s*	*s*	*c*
PROTOZOA												
ACANTHOMETRIDÆ												
78. *Acanthometron pellucidum* Muller ..	*r*	*r*	*c*	*r*	*r*	.	*r*	*f*	*f*	*r*	.	.
PROROCENTRACEÆ												
79. *Prorocentrum micans* Ehrenberg ..	*r*	.	.	*r*	.	.	.	*r*	*r*	.	.	*r*
80. *Diplosalis lenticulata* Bergh	*r*	*r*	.	.	*r*	.	.	*r*	*r*	.	*r*	.
PERIDINIACEÆ												
81. *Dinophysis miles* Cleve	.	*r*	.	.	*r*	.	*r*	*r*	.	.	.	*r*
82. *Dinophysis homunculus* Stein	*r*	.	.	*r*	.	*r*	*r*	.	.	*r*	*r*	*r*
83. *Glenodinium foliaceum* Stein	.	.	*r*	.	.	.	.	.	*r*	.	*r*	.
84. *Peridinium ovatum* Schutt	*r*	.	.	*r*	*r*	*r*	*r*	.	.	*r*	*r*	*r*
85. *Peridinium depressum* Bailey	.	*r*	.	.	.	*r*	*r*	*r*	.	.	*r*	*r*
86. *Peridinium oceanicum* Vanhoffen ..	*r*	.	.	.	*r*	.	.	.	*r*	*r*	*r*	.
87. *Ceratium tripos* Muller	*r*	.	*c*	.	*r*	*r*	*r*	*r*	*r*	*c*	*r*	*r*
88. *Ceratium massiliense* Gourret	.	.	*f*	*r*	.	.	*r*	.	*r*	*c*	*r*	*r*
89. *Ceratium breve* Schmidt	.	*r*	.	.	.	*r*	.	*r*	.	*r*	.	*r*
90. *Ceratium furca* Ehrenberg	.	.	*r*	*r*	*r*	.	.	*r*	*r*	*r*	*r*	.
91. *Ceratium fusus* Ehrenberg	*r*	.	*r*	*r*	.	.	*r*	*r*	*r*	.	*r*	.
92. *Glenodenium cinctum* Levand	.	*r*	.	.	.	*r*	*r*	*r*	*r*	.	.	.
GYMNODINIACEÆ												
93. *Polykrikos schwartzii* Butschli ..	.	*c*	*f*	.	.	.	.	*r*	*r*	*r*	.	.
CYSTOFLAGELLATA												
94. *Noctiluca miliaris* (Macartney) ..	*c*	*c*	*f*	*r*	*r*	*r*	*f*	*r*	*r*	*p*	*p*	*c*
HETEROTICHACEÆ												
95. *Stentor ræselii* Ehrenberg	*r*	.	.	.	.	.	.	.	.	*r*	*r*	*r*
OLIGOTRICHACEÆ												
96. *Tintinnus borealis* Hensen	*r*	.	.	*r*	*r*	.	.	.	*r*	*r*	*r*	.
PERITRICHICHACEÆ												
97. *Vorticella campanula* Ehrenberg ..	*r*	.	.	*r*	*r*	.	.	.	*r*	*r*	*r*	.
98. *Carchesium polypinum* Ehrenberg ..	*r*	.	.	*r*	*r*	*r*	.	.	.	*r*	*r*	.
COELENTERATA												
DIPHYIDÆ												
99. *Diphyes chamissonis* Huxley	.	*r*	*r*	.	*r*	*r*	.	.	.	*r*	*r*	*r*

TABLE II—(*Contd.*)

Name of organism	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
RHIZOPHYSALIIDÆ												
100. *Physalia utriculus* (La Martiniere) ..	.	c	.	.	.	c	.	c	f	.	p	c
CHONDROPHORIDÆ												
101. *Porpita pacifica* Lesson	r	f	r	.	.	.	.	.	.	.	p	c
102. *Velella spirans* Lesson	.	.	f	.	.	f	.	.	.	.	f	f
CHARYBDEIDÆ												
103. *Charybdæ marsupialis* Peron & Lesueur	r	.	.	r	.	.	.	r	.	.	r	.
CTENOPHORA												
PLEUROBRACHIIDÆ												
104. *Pleurobrachia globosa* Moser	c	c	r	c	r	.	f	f	f	r	c	c
BOLINIDÆ												
105. *Bolina alata* Agassiz	r	.	r	r	.	.	f	.	.	r	r	r
BEROIDÆ												
106. *Berœ flemingi* (Esch.)	r	r	.	.	.	r	f	.	r	r	r	r
ANNELIDA												
107. *Larvæ of Nereidæ*	c	f	f	c	f	r	.	.	.	.	.	.
108. *Larvæ of Eunicidæ*	.	f	.	.	.	f	c	.	.	.	c	c
109. *Larvæ of Spinoidæ*	.	c	c	c	c	f	.	.	.	.	.	.
110. *Larvæ of Terebellidæ*	f	.	.	c	.	r	.	.	.	.	.	.
CHÆTOGNATHA												
111. *Sagitta robusta* Doncaster	f	r	r	r	r	r	r	r	r	f	f	f
112. *Sagitta enflata* Grassi	c	c	f	r	r	r	.	.	f	c	p	p
113. *Sagitta tenuis* Conant	c	c	f	r	r	r	.	.	f	c	p	p
114. *Sagitta neglecta* Aida	f	r	r	r	r	r	.	.	r	f	f	f
115. *Krohnitta pacifica* (Aida)	.	r	.	.	.	.	f	f	f	c	c	c
116. *Spadella cephaloptera* (Busch) ..	r	r	r	r	r	r	r	r	r	r	r	r
POLYZOA												
117. *Cyphonautes larvæ*	r	.	.	.	.	.	.	.	.	r	r	r
PHORONIDEA												
118. *Actinotrocha larvæ*	r	r	r	r	r	r	r	r	r	r	r	r
CRUSTACEA												
CLADOCERA												
119. *Evadne tergestina* Claus	r	r	c	c	f	r	f	f	c	c	c	r
120. *Podon intermedius* (Lillj.)	r	r	.	.	r	r	.	r	.	r	.	r
OSTRACODA												
121. *Cypridina sp.*	r	r	r	r	r	r	r	r	r	r	r	r
122. *Cythere sp.*	r	r	.	.	.	r	r	.	.	.	r	r

Name of organism	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
COPEPODA												
123. *Eucalanus subcrassus* Giesbrecht ..	*f*	*c*	*f*	*r*	*r*	*r*	*r*	*f*	*f*	*c*	*c*	*c*
124. *Paracalanus parvus* Giesbrecht ..	*r*	*r*	*f*	*r*	*r*	*r*	*r*	*f*	*c*	*c*	*f*	*f*
125. *Acrocalanus longicornis* Giesbrecht ..	*f*	*f*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
126. *Centrogpages furcatus* (Dana) ..	*f*	*f*	*c*	*c*	*r*	*r*	*r*	*r*	*f*	*c*	*c*	*p*
127. *Pseudodiaptomus annandalei* Sewell ..	*r*	*r*	.	.	.	.	.	.	.	.	*r*	*r*
128. *Temora discaudata* Giesbrecht ..	*c*	*c*	*f*	*f*	*f*	*r*	*r*	*f*	*c*	*c*	*p*	*p*
129. *Acartia spinicauda* Giesbrecht ..	*p*	*p*	*p*	*f*	*f*	*f*	*c*	*c*	*c*	*c*	*c*	*c*
130. *Acartia discaudata* Giesbrecht ..	*c*	*c*	*c*	*f*	*f*	*c*	*c*	*c*	*p*	*p*	*s*	*p*
131. *Acartia erythræa* Giesbrecht	*c*	*c*	*c*	*f*	*f*	*c*	*c*	*c*	*p*	*p*	*s*	*p*
132. *Oithona nana* Giesbrecht	*c*	*f*	*f*	*f*	*r*	*r*	*f*	*r*	*f*	*c*	*c*	*c*
133. *Corycæus vensustus* Dana	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
134. *Harpacticus littoralis* Sars	*r*	*r*	.	.	*r*	*r*	.	.	.	*r*	*r*	*f*
135. *Euterpina acutiformis* (Dana)	*c*	*c*	*f*	*c*	*f*	*r*	*r*	*r*	*f*	*f*	*c*	*c*
MYSIDACEA												
136. *Rhopalophthalmus egregius* Hansen ..	*f*	*r*	*r*	*r*	.	.	.	*r*	*f*	*f*	*f*	*f*
137. *Mysidopsis kempi* Tattersal	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
138. *Heteromysis zeylanica* Tattersal ..	*r*	.	.	.	*r*	.	.	.	*r*	*r*	*r*	*r*
DECAPODA												
139. *Leucifer hanseni* Nobili	*c*	*c*	*p*	*f*	*f*	*c*	*c*	*f*	*r*	*r*	*f*	*f*
140. *Acetes erythræus* Nobili	*f*	*r*	.	.	.	*r*	.	*r*	.	.	*r*	*f*
141. *Leander styliferous* (Milne-Edwards) ..	*r*	.	.	.	*r*	*r*	.	.	.	*r*	*r*	*r*
142. *Crab-Zœa and Megalopa larvæ* ..	*f*	*f*	*f*	.	.	.	.	*c*	.	*c*	*c*	*c*
143. *Phyllosoma larvæ*	*r*	*r*	.	.	.	.	.	.	.	*r*	*r*	*r*
MOLLUSCA												
PTEROPODA												
144. *Spiratella inflata* (d'Orbingy)	*f*	*f*	*c*	*r*	*r*	.	*r*	*f*	*f*	.	*r*	*r*
145. *Spiratella trochiformis* (d'Orbingy) ..	*r*	*r*	*f*	.	.	.	*r*	*r*	.	.	*r*	*f*
146. *Spiratella bulimoides* (d'Orbingy) ..	*r*	*r*	*c*	.	.	*r*	*r*	*f*	.	.	*r*	*r*
147. *Creseis acicula* Rang	*f*	*c*	*s*	*c*	*c*	.	*p*	*c*	.	*r*	*c*	*c*
ECHINODERMATA												
148. *Bipinnaria larvæ*	*r*	*r*	*r*	*r*	.	*r*	.	.	.	.	*r*	*r*
149. *Ophiopluteus larvæ*	*r*	*r*	.	.	*r*	*r*	.	.	.	.	*r*	*r*
150. *Echinopluteus larvæ*	*r*	*r*	*r*	*r*	*r*	*r*	.	*r*	.	*r*	.	.
151. *Auricularia larvæ*	*r*	.	*r*	.	.	*r*	.	.	.	.	*r*	*r*
TUNICATA												
152. *Oikopleura cophocerca* Gegenb. ..	*f*	*r*	*r*	.	.	.	*r*	*r*	*c*	*c*	*c*	*c*
153. *Frittilaria borealis* Fol.	*f*	.	.	*f*	*r*	.	*r*	*r*	*c*	*c*	*c*	*c*
154. *Doliolum tritonis* Herdman	*r*	*r*	.	.	*r*	.	.	*r*	*r*	*r*	.	*r*
155. *Thalia democratica* (Forskal)	*r*	*r*	.	.	*r*	.	.	*r*	*r*	*r*	.	*r*
156. *Salpa cylindrica* Cuvier	*r*	*r*	*r*	.	.	*r*	.	.	.	.	*r*	*r*
ENTEROPNEUSTA												
157. *Tornaria larvæ*	*r*	*r*	.	.	.	.	*r*	*r*	.	.	.	.
PISCES												
158. *Fish eggs and larvæ*	*c*	*c*	*f*	*r*	*r*	*r*	*r*	*r*	*f*	*r*	*c*	*c*

Note.—The majority of the diatoms were kindly identified by Prof. W. E. Allen of the Scripps Institution of Oceanography, California; the algæ by Dr. M. O. P. Iyengar, formerly Director of the Madras University Botany Laboratory; the *Chaetognatha* by Dr. C. C. John, Professor of Marine Biology, Trivandrum; the *Mysidacea* by Professor W. M. Tattersal of the University of Wales; and the *Pteropoda* by Dr. Baini Prashad, formerly Director, Zoological Survey of India. The *Copepods* were identified with the help of Sewell's monograph on the Copepods of the Indian Sea (1929).

TABLE III. *Showing seasonal distribution and characters of fish eggs and larvæ of the sea around Krusadai Island*

Kind of fish	Spawning season	Diameter of egg in mm.	Chief characters of	
			Egg	Larva
Chirocentrus dorab (Forskal) ..	November to February	1·5–1·6	Frothy yolk; 7–10 colourless oil-globules; egg-membrane with a hexagonal design	54 preanal myotomes
Sardinella gibbosa (Cuv. & Val.)	September to February	0·68–0·84	Yellowish frothy yolk; one small oil-globule	29 preanal myotomes
Pellona brachysoma Bleeker ..	September to November	1·5–1·75	Broad perivitelline space; one brownish oil-globule (0·3 mm.)	Stellate black pigments near eye, otocyst and anus; 21 preanal myotomes
Dorosoma chacunda (Bloch) ..	September to December	0·95–1·2	Vacuolated yolk; about 10 small oil-globules	2·5 mm. length; chromatophores on head and myotomes; 26 preanal myotomes
Engraulis dussumieri (Cuv. & Val.)	September to December	0·85–0·95	Frothy yolk; no oil-globule	2·5 mm. length; tapering yolk-sac; brown pigments near pectoral finbuds; 17 preanal myotomes
Stolephorus indicus (V. Hasset)	June to December	1·0–1·15	Oval shaped; segmented colourless yolk; oil-globules absent	28 preanal myotomes
Dussumieria hasseltii Bleeker ..	March to December	0·88	Frothy yolk; one small colourless oil-globule at the vegetable pole	Anus under 45–48 myotome; fine black pigment spots, scattered along the myotomes

Chanos chanos (Forskal) ..	March to August	1·1–1·2	Yellowish frothy yolk	Transparent elongated body; 31–33 preanal myotomes; black pigments over finfold
Therapon jarbua (Forskal) ..	October to December	0·4–0·5	Clear perivitelline space	10 preanal and 15 post-anal myotomes
Otolithus ruber (Bl. Schn.) ..	August to September	0·5–0·7	Dark and granular yolk	..
Trichiuris savala (Cuv. & Val.)	September to October	2·15–2·4	One yellow oil-globule (0·7 mm.)	Paired black pigments near ear vesicles; 34 preanal and 126 post-anal myotomes
Caranx hippos (Linn.) ..	August to September	0·7–0·9	Segmented yolk; one yellowish oil-globule (0·18 mm.) dark pigments	1·6–1·8 mm. in length; brown pigments on dorsal side of body; 12 preanal myotomes
Leiognathus bindus (Cuv. & Val.)	November to January and April to June	0·4	Yellow frothy yolk; broad perivitelline space	10–20 mm. in length; transparent body; black pigments on head
Cybium commersonii Lacep. ..	May to July	1·0–1·25	One large oil-globule (0·3 mm.); several stellate chromatophores on yolk and oil-globule	2·2–2·5 mm. in length; black pigments along vertebral column; 13 preanal myotomes
Sillago sihama (Forskal) ..	November to March	0·5–0·6	Little perivitelline space; no oil-globule	Pigments near dorsal and anal fins; pointed mouth
Mugil seheli (Forskal) ..	November to February	0·48–0·55	Granulated yolk; no oil-globule	Opaque white in colour

The diatom flora of Krusadai is found to be similar to that of the Java Sea described by Allen and Cupp (1935) of the Trivandrum coast by Menon (1945), and of the Madras Coast by Subrahmanyam (1946). A noticeable characteristic is the variety of diatoms that is usually found in swarms. The common and important diatoms are species of *Coscinodiscus*, *Rhizosolenia*, *Chætoceras* and *Thalassiothrix*; and they occur almost throughout the year. The other species are also found at most times of the year but are never so abundant. *Biddulphia* stated to be abundant off the Madras Coast by Menon (1931) is comparatively rare. The period of maxima of the diatom population is usually from June to November, when there is a rise in salinity and temperature of the water. In the West Coast, on the other hand, Chidambaram and Menon (1945) have observed that the phytoplankton is rich in the months of July, August and September, when the surface temperature and specific gravity are low. For a closer understanding of these, we have to obtain more precise hydrological data than is now available.

The permanently planktonic blue green alga, *Trichodesmium erythræm*, is very common, and is seen in dense swarms during the months of April, May, July, October and November. When it gathers at the surface in swarms the sea is coloured brownish yellow and emits strong smell of chlorine. On such occasions, which usually last 3 to 7 days, heavy mortality of marine organisms takes place. Such inhibitory and lethal effects of *Trichodesmium* have already been reported at Krusadai by Chacko (1942) and by Chidambaram and Unny (1944) and similar instances have also been reported by John and Menon (1942) from the Travancore coast, and by Delsman (1939) from the Java Sea.

Following the succession of diatoms, it cannot be said that there is any definite intensities corresponding with the protozoans occurring here. The ' red water ' phenomenon associated with dinoflagellates periodically recorded from the Malabar coast by Hornell and Nayudu (1923), from Madras by Aiyar (1936) and from Travancore by Menon (1945) have not been observed in the Krusadai waters during the period of this study. *Noctiluca miliaris* is common from October to February but its inhibiting influence on other organisms on the West Coast recorded by Devanesen (1942) has not been noticed here probably owing to the fact that the swarms do not attain same high density.

The Hydromedusæ, Siphonophores and Ctenophores are fairly represented throughout the year, being common from November to February, when the sea is usually characterised by strong currents. As the polychæte

fauna of the island and its littoral area is a rich and varied one, it is not surprising that there is such a large variety of them in the plankton with a maximal period from January to May.

The Chætognaths are present throughout the year but larger numbers are encountered during the cold weather, from November to February. *Krohnitta pacifica* and *Spadella cephaloptera* were recorded for the first time from this area by Varadarajan and Chacko (1943). The Chætognaths, which play an important role in the food cycle of sea, prey on diatoms, ciliates, copepods, larval fish, and are sometimes cannibalistic. A large variety of animals have been found to prey upon these and they have been also found in the stomach contents of plankton feeders like *Sardinella gibbosa* (Cuv. and Val.) and *Dussumieria hasseltii* (Bleeker).

The Copepods form the most important members of the pelagic fauna, averaging about 40 per cent. of the total zooplankton population. The hydrographical conditions of this area during their maximal period (October to March) may therefore be inferred to be favourable for their multiplication.

A systematic study of the pelagic eggs and larvæ of important fishes is a problem of considerable economic importance. In the plankton of the sea around Krusadai Island, fish eggs and larvæ are observed in almost in every month, indicating thereby that this area is probably a suitable spawning ground for many species. Eggs and larvæ of 17 species have been identified from this region, the details of which are given in Table III, with the help of the descriptions of Delsman (1924–30) on the fish eggs and larvæ from the Java Sea.

V. Acknowledgments

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LERNAEOPODA STROMATEI N. SP., A COPEPOD PARASITE OF THE GREY POMFRET

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Several copepods parasitic on the grey pomfret (*Stromateus niger*) have been described already by Steenstrup and Lutken, Heller, Bassett-Smith, Kirtisinghe and others. Among the Lernæpodidæ, *Thysanote appendiculata* which has been recorded by Steenstrup and Lutken (1861), Bassett-Smith (1898), Kirtisinghe (1937) and the author, belongs to the sub-family clavellinæ. The present form *Lernæpoda stromatei* n. sp., belongs to the lernæopodinæ, a sub-family not recorded before from India. In 1915 when Wilson revised all the known species of the genus *Lernæopoda*, he established new genera to receive those which do not naturally belong to the genus and left finally five species. To these the following four species have been added: *L. Scyllicola* Leigh Sharpe (1916), *L. bivia.* Leigh Sharpe (1930), *L. obesa* Brian (1924), and *L. etmopteri* Yamaguti (1939). Unlike the nine known species which are parasitic on the plagiostomi, the present form is parasitic on the pomfret and differs from them in a number of specific characters. Hence it is treated as a new species and a full account is given here.

Host and record.—While examining nearly a score of the pomfrets, *Stromateus niger* of different sizes caught on the Madras shore between September and November, three parasites were collected from the gill arches of two fishes. Of these parasites one was a male which had just become mature while the other two were females with out egg sacs, one being slightly older than the other. The more mature female is treated as the holotype and will be lodged in the Indian Museum.

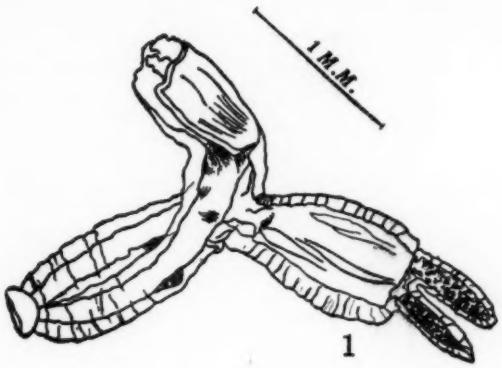
The Female

Size and colour.—The parasite is yellowish white in colour the cuticle being transparent, loose and wrinkled. Exclusive of the posterior processes which were 0·52 mm. long, the parasite measured 2·1 mm.; the second maxilla was 1·54 mm. the cephalothorax 1·05 mm., the trunk 1·08 mm. The trunk and cephalothorax were 0·61 mm. broad, inclusive of the inflated cuticle.

General appearance of body.—The cephalothorax is circular, flattened dorso-ventrally and covered with a distinct carapace. The carapace is arched above with the straight-edged sides bent downwards and the front edge broader than the hind margin. The first two segments to which the maxillipedes and the second maxillæ are attached, are much narrower and form a neck which is inclined to the trunk. The trunk is oval, broadest about a fourth of the length from behind and tapers to where it joins the cephalothorax. The posterior convex margin is marked ventrally by a spherical short-stalked genital process. On either side of, and dorsal to which are attached two posterior processes. These are conical, tapering at either end. The trunk is not segmented. Though there is no recognisable abdomen as such, yet, the persistence of anal laminæ even in forms like *Clavellisa dussumieria* suggests that the "trunk" represents the genital segment as well as the fused abdomen.

Appendages.—The first antenna is uniramous, four-articled. The distal segment is longer, narrower and bears a pointed process subterminally and three other processes as well as a rounded lobe terminally. The second antennæ sweep out on either side of the head, anterior to the first antennæ and curve medially with their tips almost meeting each other, dorsal and anterior to the mouth-tube. Each antenna is biramous and has a stout, long two-jointed protopod. The exopod is conical and has a tip of imbricated lobes and a short slender process while the endopod is two-jointed and bears a pair of small claw-like spines. The mouth tube is conical and has its free tip covered with slender pápillæ. Through the ventrally directed mouth can be seen the tips of the mandibles, bearing seven large obtusely pointed teeth. The first maxilla is tripartite bearing three dactylose acute-tipped lobes. The palp is shorter and has two spines.

The second maxillæ commence from close behind the maxillipedes. They are longer than the cephalothorax or the trunk. They extend to the point of attachment separately and are united only at the tips. They bear two glandular swellings, the maxillary glands, in the first third of their length. The fused tips of the maxillæ show two separate discs or thick swellings below the broadened base of the manubrium. The fused part which is raised into a collar round the manubrium, is without any armature. The distal tip of the manubrium is knobbed and buried in the button-shaped bulla whose rim is thin and broadened. The two canals from the maxillæ extend unbranched and separate into the bulla. The maxillipedes arise immediately in front of the second maxillæ, both being close behind the mouth tube. There is a stout muscular basal portion to which is articulated a long tapering joint



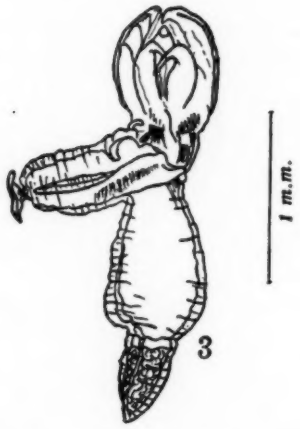


FIG. 1. Dorsal view of female *Lernæopoda stromatei*
FIG. 3. Immature female

bearing a strong primary claw as well as a thinner secondary spine. The basal joint also bears on its inner side a short apine.

THE MALE

A solitary specimen was found attached by its larval filament to the gill arches, close to the female. This form is a mature male which has not yet become attached to the female. As Wilson had found in the case of *Actheres ambloplitis*, the swollen free end of the filament was gripped by the short blunt claws at the tips of the second maxillæ. The second maxillæ were elongate cylindrical and held at right angles to the body.

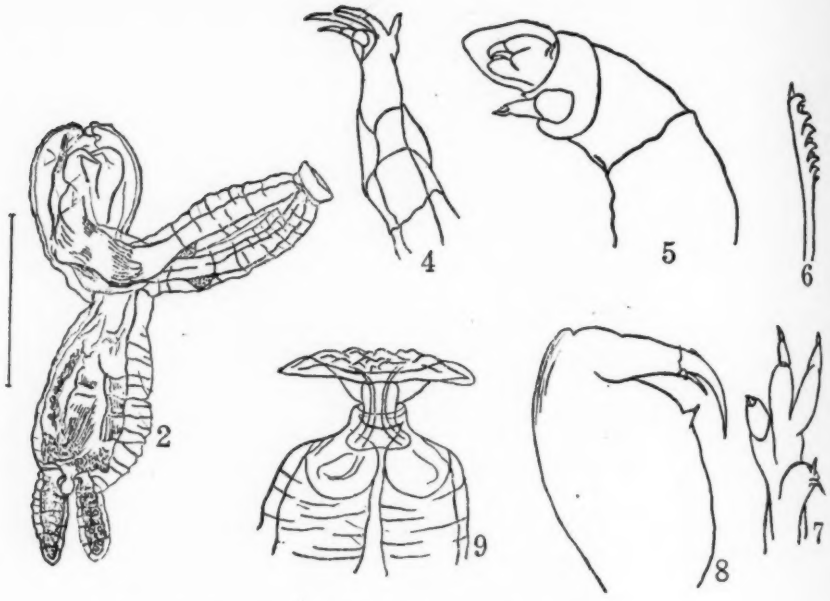


FIG. 2. Ventral view of female *Lernæopoda stromatei*
FIG. 4. First antenna
FIG. 5. Second antenna
FIG. 6. Mandible
FIG. 7. First maxilla
FIG. 8. Maxillipede
FIG. 9. Bulla

The body measured 1·2 mm. exclusive of the anal laminæ. Proportionate to the size of the female (2·1 mm.), the size of the male is large as has been observed in other species of the genus. The cephalothorax which is 0·58 mm. long, is slightly shorter than the trunk. There is no carapace descernible in this form. The region where the maxillipedes and the second maxillæ were attached appeared distinct and well marked from the anterior part of the cephalothorax. A definite constriction separated the trunk behind. The trunk was oval and elongated with but three faint grooves dividing the the body into four segments. When cleared in xylol and mounted in balsam, these grooves became indistinct. The pair of lanceolate anal laminæ were so articulated to the conical posterior tip of the trunk that they appeared capable of being turned ventrally or dorsally.

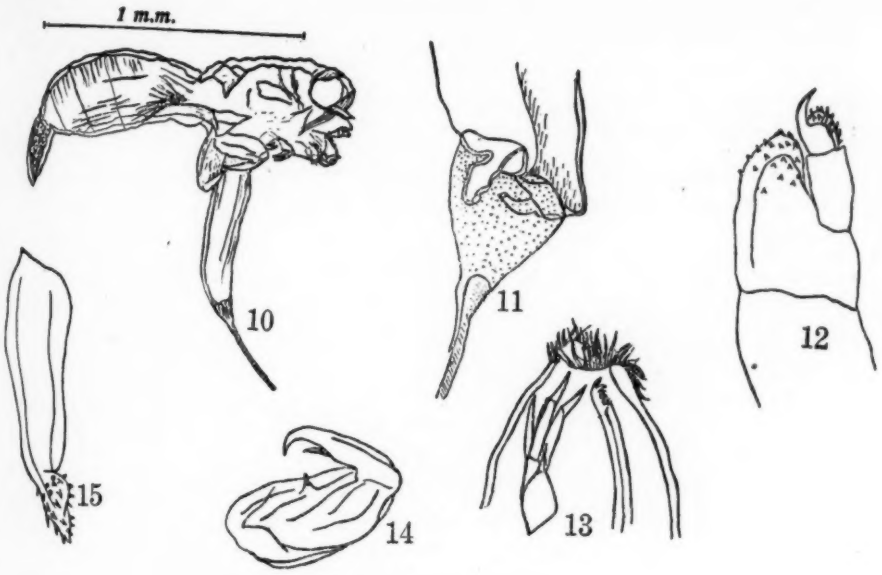


FIG. 10. Mature male of *Lernæopoda stromatei*
FIG. 11. Tips of the second maxilla gripping the base of the filament
FIG. 12. Second antenna
FIG. 13. Mouth cone mandibles and first maxilla
FIG. 14. Maxillipede
FIG. 15. Anal lamina

Appendages.—The first antenna is long slender and tapering. It is directed anteriorly under the thickened rounded end of the frontal region. It is four-articled, the distal segment being marked by a pad as well as a curved spine. The second antenna, though stouter and longer, lies behind. It is biramous. The stout protopod tapers to bear the rami. The exopod which is longer, is two-jointed and bears a stout sickle-shaped claw at the base of which are borne two or three straight spines. The distal joint bears a number of minute spines. The mouth cone is long and narrow. Its distal end as well as the edge of the mouth bear whorls of papillose spines. Through its transparent walls the long slender mandibles can be seen. Each mandible has a flattened free tip bearing seven teeth of unequal size. The first maxilla is tripartite, two lobes being longer than the third. The palp is much shorter and is seen close to the base. The second maxillæ, which together with the maxillipedes are only a little removed behind, are as long as the cephalothorax. Each maxilla is columnar, straight and tipped by a cushion-like pad, provided

with a stout curved claw. As the tips of the maxillæ come together, the claws grip the swollen base of the larval filament. The maxillipedes which are posterior to the maxillæ are of the same type as in the female. Each bears a mamillated spine on its proximal joint as well as primary and accessory spines on the distal joint.

The anal laminæ are long and have a well marked terminal part bearing a large number of aborted spines.

Remarks.—All the nine species of the genus, established up to date, appear to be parasitic on plagiostomous hosts. Of these only *L. bivia*, *L. scyllicola*, *L. musteli*, *L. galei* and *L. elongate* have a button-shaped bulla, while the maxillæ are longer than the trunk in *L. musteli*, as in the present form. *L. musteli* Thomson which appears to have been far larger than the present form was found parasitic within the cloaca of *Mustelus antarcticus* from New Zealand. Further from the scanty data available about this species, Brian has thought it justifiable to transfer it to the genus *Actheres* and Wilson has retained it in the genus *Lernæopoda* provisionally. Owing to these differences, the present form is described as a new species. As the male described in this paper cannot be strictly treated as a type, this Species *Lernæopoda stromatei* is described with only the female as the holotype and can be defined as follows:—Cephalothorax is flattened dorsoventrally, circular in outline. The first two segments forming a neck inclined at an angle with the trunk, bear the maxillipedes and the maxillæ close to the mouth. The second maxillæ are longer than the trunk, united at the tips. The bulla is button-shaped. There is a spherical genital process and two conical posterior processes on the flask-shaped trunk.

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STRAW MANURING IN RELATION TO AMYITPO DISEASE OF RICE

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Introduction

A nutritional disorder of rice, locally known as *amyitpo* (literally, root disease), was found to occur in the vicinity of Zegyogon village in Patheingyi township, near Mandalay, Upper Burma in 1938. Subsequently the same disorder was observed at Nyaungbintha in Mandalay Canal area, and at Singaing, south of Mandalay.

In preliminary manuring experiments conducted at Zegyogon village during 1938 and 1939 it was found that the disease could not be controlled in the field by the use of calcium sulphate, Nicifos II, secondary calcium phosphate or superphosphate. The presence of eelworms in the diseased plants suggested that they might be the cause of the disease. The usual treatments, namely, zinc sulphate at 50 pounds per acre or calcium cyanamide at 3 cwt. per acre, applied to the soil proved to be ineffective. Moreover, the healthy plants in the area also contained eelworms. The fungus *Sclerotium Oryzæ* found in abundance on the deceased plants suggested the possibility that this might be the primary cause of the trouble. This suggestion became untenable when the diseased plants were found to recover on manuring with potassium sulphate. The probability was that the diseased plants possessing an abnormal composition became an easy prey to fungal invasion.

Observation during the growing season showed that the disease was localised and appeared in its worst form in depressions and in patches surrounding the water outlets. Areas outside these patches and some of the adjacent fields, however, produced normal crops (Plate VIII *a*).

Soil collected from the affected patches in fields at Zegyogon village, Patheingyi township (Patheingyi soil) was examined in the laboratory during 1940 when it was found that the soil was a heavy clay with a pH of 8·15 and that there was no absolute deficiency of nutrients in the soil as judged by laboratory methods. On the other hand, rice plants grown in the soil in pots showed very striking symptoms which were found to be due to acute potassium deficiency. The plants responded strongly to treatment

with potassium sulphate though there was fair response to ammonium sulphate also (Plate VIII *b*). These results were discussed fully in a recent article (Aiyar, 1948). It was concluded therein that potassium deficiency in Patheingyi soil was caused partly by ionic antagonism but mainly by assimilation of available potassium by soil micro-organisms.

The organic matter of Patheingyi soil was found to have a wide carbon-nitrogen ratio, a condition favouring an abnormal development of micro-organisms. The most important contributory factor was found to be the presence of undecomposed straw which had accumulated in lowlying areas in the field. This accumulation was found to occur during cultivation, especially harrowing, when the stubbles left from the previous season's harvest were moved about and left in depressions. This local difference had to be taken into account in order to explain the origin and occurrence of *amyitpo* disease in these lowlying areas.

In a review of the harmful effects of straw manuring Collison and Conn (1925) conclude that the main effect is a nitrogen starvation of the crop though some depressive action is also attributed to an organic constituent of straw. Although the effects of straw are generally understood to be due to increased bacterial development, attention has been directed by Thom (1935) to the exceptionally high numbers of bacteria produced by the addition of bulky organic materials to the soil. The superior feeding powers of bacteria in competition with the growing plant have been stressed by Doryland (1916) and Russell (1942).

Mc Intire and Sanders (1930) showed that the potassium applied in the form of green manures was fixed in the soil in non-available form in the presence of liming materials. Jenny and Shade (1934) suggested that such fixation was due to assimilation by bacteria. Biological fixation of potassium in soil has been confirmed by many workers (Dean, 1936; Den Doop, 1941; Hurwitz and Batchelor, 1943).

Espino and Pantaleon (1934) found that the chlorosis induced in rice seedlings by straw manuring could be prevented by allowing the straw to decompose in the soil for a sufficiently long period before planting. A similar chlorosis caused by cane bagasse was prevented by applying an equivalent quantity of its ash (Malabayabas, 1934).

The potassium present in straw has been found to become available to crops in soils rich in nitrogen (Sears, 1930; Lamb, 1935).

Collison and Conn (1922) showed that partial sterilisation with carbon disulphide of a soil treated with straw led to a large increase in crop yield. Rogers (1940) found that acriflavine was very effective in suppressing the

development of sulphate-reducing bacteria in soil. The author successfully used acriflavine in overcoming the bacterial fixation of phosphorus applied to a phosphate-deficient soil in the form of raw rice bran (Aiyar, 1946).

The action of rice straw was investigated from various aspects in order to elucidate the causes of *amyitpo* disease. The results obtained in this work are described in the present article.

Experimental

The experimental work consisted of (1) pot experiments with Patheingyi soil to study the effect of straw, (2) examination of plant samples from fields in *amyitpo* areas, (3) water culture experiments, and (4) demonstrations.

A. Experiment to Study the Effect of Straw

These experiments were carried out in conjunction with manuring experiments on Patheingyi soil (Aiyar, 1948). The treatments are shown in Table I. One week after transplanting, the straw was added as a coarse

Table I. *The Effect of Straw and Other Treatments on Growth and Yield of Rice in Patheingyi Soil*

Treatments	Height of plants on 15–11–40 cm.	Length of leaf-blade on 15–10–40 cm. Blade No.				No. of tillers			No. of panicles on 15–11–40	Appearance of plants at harvest on 16–12–40	Yield of crop Total of 4 pots		Straw / Grain ratio
		1 oldest	2	3	4	15–9–40	15–10–40	15–11–40			Straw grs.	Grain grs.	
A. 1 ..	71	28	33	27	..	4	4	9	2	Green	21·5	4·7	4·5
A. 2 ..	74	30	32	27	16	3	3	10	4	Green	20·4	5·7	3·6
A. 3 ..	125	55	57	59	61	8	16	12	12	Ripe	120·1	85·3	1·4
A. 4 ..	94	35	37	35	27	3	6	14	5	Green	49·7	33·5	1·5
A. 5 ..	80	38	32	35	32	2	4	6	4	Green	21·0	3·9	5·3
A. 6 ..	120	48	55	60	..	5	11	13	13	Ripe	83·6	68·8	1·2
A. 7 ..	62	28	28	30	..	2	3	4	2	Green	10·5	2·0	5·2
A. 8 ..	109	44	49	55	..	4	11	14	13	Ripe	79·2	66·1	1·2

Critical difference between two total yields for significance at 1 per cent. level — Straw — 26·3; Grain — 5·4

Key to Treatments

A. 1. Untreated.
A. 2. Ten c. c. of acriflavine solution only.
A. 3. Potassium sulphate at 0·3 g. of K_2O per pot.
A. 4. Straw — 10 g. per pot.
A. 5. Straw — 25 g. per pot.
A. 6. Straw — 25 g. per pot plus 10 c. c. of acriflavine solution.
A. 7. Straw — 50 g. per pot.
A. 8. Straw — 50 g. per pot plus 10 c. c. of acriflavine solution.

powder to the puddled surface layer of soil in the pot and mixed with the mud using a glass rod for the purpose. Ten c.c. of acriflavine (0·02 per cent. in water) were added along with the straw when this antiseptic was used. When straw was added at 100 g. per pot the rice seedlings rotted and died. The rate of application was therefore kept well below this limit.

Observations taken during the growth period as well as the yields of straw and grain are shown in Table I, and the chemical composition of the crop in Table II.

TABLE II. *Composition of Rice Plants grow in Patheingyi Soil with Various Treatments as shown in Table* I

(Percentages on oven dry basis)

Treatments	Straw							Grain		
	N	P_2O_5	K_2O	CaO	MgO	Fe_2O_3	Mn	N	P_2O_5	K_2O
A. 1 ..	1·512	0·320	1·304	0·265	0·608	0·204	0·016	1·771	0·724	0·248
A. 2 ..	1·490	0·372	1·000	0·291	0·544	0·144	0·018	1·659	0·705	0·262
A. 3 ..	0·322	0·095	2·818	0·191	0·336	0·024	0·018	0·945	0·611	0·363
A. 4 ..	1·267	0·322	2·070	0·224	0·576	0·220	0·020	1·757	0·743	0·283
A. 5 ..	1·701	0·441	1·792	0·304	0·672	0·212	0·022	1·862	0·782	0·202
A. 6 ..	0·357	0·098	3·068	0·224	0·384	0·063	0·018	1·057	0·633	0·327
A. 7 ..	2·345	0·491	1·813	0·336	0·840	0·208	0·038	1·925	0·775	0·210
A. 8 ..	0·420	0·120	3·372	0·190	0·320	0·068	0·018	0·945	0·645	0·367

The straw used in these experiments contained 2·0 per cent. of potash so that the quantities of straw used contained 0·2 g., 0·5 g. and 1·0 g. respectively of potash per pot. The effect of straw may thus be compared with that of potassium sulphate which was applied at the rate of 0·3 g. of potash per pot.

Inspection of the data presented in Tables I and II reveals the following features:—(1) a small amount of straw produced a moderate increase in yield of grain and straw in normal proportions though the crop was immature and of abnormal composition, (2) increase in rate of application of straw caused a sharp reduction in yield of crop which was practically identical with the unmanured crop in being immature, with a wide straw-grain ratio and abnormal composition, (3) in the presence of acriflavine, straw treatment, even in large doses, did not produce any depressive action but produced a large increase in yield of mature crop of narrow straw-grain ratio and of normal composition, (4) treatment of the unmanured soil with acriflavine did not produce any effect and (5) straw manuring produced

various other symptoms such as progressive stunting of the plant and leaf-blades, delayed formation of tillers, reduction in the proportion of fruitful tillers and failure to reach maturity.

The various symptoms of potassium deficiency pointed out elsewhere (Aiyar, 1948) were thus identical with those produced by straw manuring but the symptoms did not appear when acriflavine was added as an antiseptic along with the straw (Plate IX *a* and *b*).

B. Further Experiments with Straw

Since the potassium contained in straw was found to be available to the rice crop in the presence of acriflavine other methods for improving the availability of the potassium present in the straw were examined. The following methods were tried: (1) partial sterilisation of the soil by heating with steam for one hour, (2) ashing the straw and adding the quantity of ash which corresponds to the straw to be used, and (3) allowing the straw to decompose in the waterlogged soil for various periods and, where stated, the decomposition was supplemented by drying the soil-straw mixture and exposing it to the sun for a week. Puddling and waterlogging were carried out as usual. Each pot received one seedling. There were four replications for each treatment. The treatments together with the corresponding yields and chemical composition of the crop are shown in Table III.

From the data of Table III the following conclusions may be drawn: (1) partial sterilisation of the soil has caused a large increase in yield, the crop being ripe and normal in composition; (2) although fresh straw is injurious it becomes progressively beneficial when buried in the waterlogged soil for long periods and it appears that the decomposition must proceed for a minimum period of three months before the crop becomes normal in appearance and composition; (3) even after burial for four months in waterlogged soil the harmful effect does not disappear entirely as may be seen from the increased yields obtained by drying the soil after preliminary decomposition; (4) treatment with straw ash is beneficial and as good as treatment with fully decomposed straw though much inferior to potassium sulphate treatment; (5) the effects produced by straw treatment are identical with those produced by potassium deficiency in Patheingyi soil, namely, low yields characterised by a wide straw-grain ratio, failure of the crop to ripen, and abnormal composition of the crop involving high percentages of nitrogen and phosphoric acid with correspondingly low percentages of potash.

TABLE III. *Yield and Composition of Rice Plants grown in Patheingyi Soil and Manured with Straw in Different Ways*

Treatment No.	Total yield of 4 pots : grams		Straw / Grain ratio	Appearance of crop at harvest	Composition of straw : percentages on oven-dry basis		
	Straw	Grain			N	P_2O_5	K_2O
B. 1 ..	16·1	3·6	4·47	Green	1·686	0·342	1·008
B. 2 ..	55·8	42·7	1·37	Ripe	0·465	0·216	2·818
B. 3 ..	96·3	67·6	1·42	Ripe	0·385	0·127	2·986
B. 4 ..	9·2	2·2	4·20	Green	2·421	0·465	1·675
B. 5 ..	15·6	5·8	2·70	Green	1·965	0·386	1·816
B. 6 ..	37·9	22·7	1·70	Green	0·984	0·284	2·032
B. 7 ..	63·8	39·3	1·62	Ripe	0·442	0·167	2·915
B. 8 ..	71·4	49·5	1·44	Ripe	0·403	0·122	3·181
B. 9 ..	88·6	61·1	1·45	Ripe	0·376	0·119	3·018
B. 10 ..	68·8	46·2	1·49	Ripe	0·388	0·120	3·073
B. 11 ..	117·6	80·6	1·47	Ripe	0·345	0·105	3·155

Critical difference between two total yields for significance at one per cent. level — Straw — 24·6; Grain — 10·8

KEY TO TREATMENTS

B. 1. Unmanured, puddled and transplanted.
B. 2. Soil heated with steam for one hour, puddled and transplanted.
B. 3. Soil heated with steam for one hour, puddled and treated with 50 g. of straw per pot at the time of transplanting.
B. 4. Soil puddled and treated with 50 g. of straw per pot at the time of transplanting.
B. 5. Soil puddled and treated with 50 g. of straw per pot and kept waterlogged for one month before transplanting.
B. 6. As in B. 5 but waterlogged for two months before transplanting.
B. 7. As in B. 5 but waterlogged for three months before transplanting.
B. 8. As in B. 5 but waterlogged for four months before transplanting.
B. 9. As in B. 8 but after waterlogging for four months the soil was dried, pounded, sundried for one week, and again puddled before transplanting.
B. 10. Soil puddled and treated with straw ash corresponding to 50 g. of straw per pot at transplanting.
B. 11. Soil puddled and treated with potassium sulphate at 0·3 g. of K_2O per pot at transplanting.

C. *Analysis of Field Samples from* Amyitpo *Areas*

During the growing season of 1940–41 several samples of *amyitpo* affected as well as healthy rice plants from adjacent areas were collected on the 15th of October (Plate III *a*). The samples were compared in the field and afterwards analysed. The data obtained on these samples are shown in Table IV.

When examined in the field the healthy plants were normal in appearance though somewhat taller than usual. On the other hand, the diseased

TABLE IV. *Composition of Field Samples of Rice Plants from* Amyitpo *Areas*

Localities and nature of samples	Height of plants cm.	Colour of plants on 15-10-40	Composition of tops of plants (percentages on oven-dry basis)						
			N	P_2O_5	K_2O	CaO	MgO	Fe_2O_3	Mn
ZEGYOGON—									
1(*a*) Healthy ..	166	Yellowish green	1·48	0·22	4·05	0·53	0·41	·021	·019
1(*b*) Diseased ..	48	Bluish green	2·58	0·42	2·33	1·06	0·55	·255	·063
2(*a*) Healthy ..	154	Yellowish green	1·59	0·30	3·27	0·42	0·33	·016	·028
2(*b*) Diseased ..	53	Bluish green	2·76	0·49	1·16	0·62	0·45	·189	·071
NYAUNGBINTHA—									
3(*a*) Healthy ..	159	Light green	1·13	0·28	3·75	0·40	0·41	·019	·014
3(*b*) Diseased ..	44	Bluish green	2·08	0·38	1·42	0·55	0·68	·222	·048
4(*a*) Healthy ..	175	Light green	1·07	0·33	4·18	0·51	0·38	·012	·018
4(*b*) Diseased ..	64	Bluish green	2·27	0·42	1·95	0·63	0·59	·168	·057

plants were highly stunted, squat shaped and of a bluish green colour. The older leaves and leaf-sheaths showed extensive wet rot. There were purple patches on the leaves of some of the diseased plants. There was no tendency to show any yellowish tinge; the leaves of the diseased plants showed abnormal stiffness and many tended to stay at right angles to the stem. There was also progressive shortening of the leaves from the oldest to the youngest.

In comparison with the healthy plants, the *amyitpo*-affected plants showed much lower percentages of potash and correspondingly higher percentages of nitrogen, phosphoric acid, lime and magnesia. The accumulation of iron in the diseased plants was noteworthy.

The symptoms and abnormal composition of *amyitpo*-affected plants are therefore identical with those found in cases of potassium deficiency.

D. Water Culture Experiments with Amyitpo-*affected Rice Plants*

In preliminary experiments using the three-salt solution of Gregory and Richards (1929) as standard it was found that specimens of *amyitpo*-affected rice plants taken from the field recovered and grew to maturity in the complete culture solution and in the solution lacking in nitrogen. On the other hand, in solutions lacking in phosphorus or potassium (but containing nitrogen) the *amyipto* plants died without recovery. This result was apparently due to the fact that the diseased plants were already too rich in nitrogen. Recovery was rendered difficult as the transfer to the culture solutions was made somewhat late in the season when the disease had seriously affected the plants. However, since even the healthy plants produced very little grain in the complete solution it appeared probable

that the composition of the culture solution was not suitable. These data are shown in Table V (*a*).

Another experiment was therefore carried out using a modification of Kuilman's solution (Aiyar, 1948). The plants were removed from the field on the 20th of August when the diseased condition had become evident but had not advanced too far. The data of this experiment are shown in Table V (*b*). From these data it became further confirmed that the *amyitpo* disease was caused by potassium deficiency. Even the originally healthy plants acquired the characteristic features of potassium deficiency when grown in the solution lacking in potassium, namely, the bluish-green colour, the tendency to suffer premature decay without turning yellow, and poor grain formation.

TABLE V. *Yield of Dry Matter of Rice Plants from* Amyitpo *Area Transferred to Culture Solutions on Dates as Shown*

Grams — Mean of Three Bottles

Treatment	Healthy plants			*Amyitpo* Plants		
	Root	Straw	Grain	Root	Straw	Grain
(*a*) 30–9–39 : First Series						
Complete solution ..	5·7	17·4	1·6	4·7	7·3	1·0
– N ..	6·8	9·7	0·5	1·8	3·4	0·1
– P ..	4·3	11·3	0·3	– Plants	Died	..
– K ..	9 7	22·0	1·9	– Plants	Died	..
(*b*) 20–8–40 Second Series						
Complete solution ..	7·7	25·9	10·8	4·3	16·8	6·3
– N ..	6·3	42·5	9·3	3·7	12·9	4·3
– P ..	5·2	18·3	10·5	3·6	7·1	3·5
– K ..	2·9	7·6	1·8	– Plants	Died	–

E. Demonstrations

Since the *amyitpo* disease had been found to be a nutritional disorder caused by potassium deficiency it was decided to carry out a few simple demonstrations in the field during the 1941 season. Large-scale experiments were not possible owing to the shortage of the fertiliser.

Fifty gram lots of finely powdered potassium sulphate were stirred into the mud on 20–8–41 in each of ten small plots where the *amyitpo* disease had appeared. When examined on 15–9–41 the treated plants had all recovered. They produced normal earheads in November and ripened satisfactorily by 15–12–41 when the plants were harvested (Plate X *b*).

Discussion

(*a*) *Straw in relation to Microbial Development*

The symptoms of *amyitpo* disease of rice have now been completely identified with those produced by potassium deficiency (data of Tables I, II and III). It was suggested elsewhere (Aiyar, 1948) that the deficiency was caused by abnormally high microbial development in the soil. Various authorities (Thom, 1935; Russell, 1942) have pointed out that bulky organic materials added to soil may suffer decomposition of an "explosive" character and that bacterial numbers may rise up to 50,000 millions per gram of soil. It has been estimated that Patheingyi soil may contain at least 20,000 millions of bacteria per gram. The presence of 3·2 per cent. of calcium carbonate in this soil makes it a very favourable medium for bacterial development. The original cause of the abnormal development of micro-organisms in Patheingyi soil was traced to the stubbles of straw which are ploughed into the soil every year. The material actually accumulates in depressions and areas surrounding the water outlets in the field. It is precisely in these areas of straw accumulation that the *amyitpo* disease occurs characteristically.

(*b*) *Straw as a Source of Potash*

Since rice straw may contain up to 3·0 per cent. of potash, mostly soluble in water, the development of potassium deficiency by manuring with straw must obviously be attributed to the high proportion of carbohydrates present in straw. The beneficial effect of a small dose of straw and the progressive depression produced by increasing dosage (Table I) are in accordance with these facts. In soils rich in nitrogen it has been observed that straw may serve as a source of potash (Sears, 1930; Lamb, 1935). The author found (Aiyar, 1948) that manuring the Patheingyi soil with ammonium sulphate led to an increase in potassium availability to the rice crop in pot culture, though the action was subject to delay.

From a study of the conditions under which straw serves as a source of nutrient, be it nitrogen, potash, phosphoric acid or any other, it is obvious that the conditions have to be so adjusted that abnormal development of micro-organisms is prevented, but if the development is unavoidable or has already taken place it is essential to break down the organic matter by providing adequate nitrogen, by controlled heating or by other means. The use of straw as a mulch may cause less trouble than its incorporation into the soil. The application of the ash of straw is another useful device. This result may be achieved by burning the stubble in the dry season, a common practice in Lower Burma, though not in Upper Burma.

The beneficial effect of straw ash may be seen in the data of Table III.

Treatment with carbon disulphide of soil manured with straw has been found to give large increases in yield of crop (Collison and Conn, 1922). Under waterlogged conditions a soluble antiseptic such as acriflavine was considered to be more suitable than carbon disulphide. Rogers (1940) found that one part of acriflavine in 750,000 parts of water suppressed the development of sulphate-reducing bacteria in soil. In the present work acriflavine was applied to the surface water in pots to give a concentration of one part in 50,000 after the straw was incorporated into the mud. This treatment was very effective as the potassium present in the straw became readily available to the crop leading to ripening and normal composition of the crop (Tables I and II). It may be mentioned, however, that acriflavine was ineffective in releasing potassium from unmanured Patheingyi soil (Table II).

Partial sterilisation of a soil by steam heating is generally assumed to increase the available nitrogen though nothing specific seems to be accepted about the availability of potassium (Russell, 1942). The results reported in Table III show that heating the soil with steam has been quite effective not only in making available the potassium contained in straw but also in releasing potassium from the unmanured soil where it had apparently been retained in insoluble form in microbial cells.

As a means of overcoming the harmful effect of straw it was buried in the soil and allowed to decompose for different periods. Under waterlogged conditions the decomposition was slow and incomplete even after four months as judged by the crop response (Table III).

It may be concluded that straw manuring is definitely harmful to the rice crop in Patheingyi soil as it gives rise to *amyitpo* disease due to potassium deficiency. As a sanitary measure it is probably best to burn off the stubble after the harvest. This procdedure also ensures the return of the potash contained in the straw to the soil. Methods employing partial sterilisation or antiseptics in conjunction with undecomposed straw are not likely to be practicable on a field scale for the recovery of potash. These indirect methods are, however, scarcely to be recommended for the control of *amyitpo* disease, as in fact, the direct application of potassium sulphate has been found to be much more effective in Patheingyi soil.

Summary

1. The symptoms of *amyitpo* disease of rice produced in Patheingyi soil are shown to be identical with those of potassium deficiency.

2. The part played by straw as a cause of the disease was investigated and it was found that straw depressed the growth of rice in proportion to the rate of application. With the largest application the crop rotted and died. The smallest rate of application caused a moderate increase in yield. Symptoms of potassium deficiency were produced in each case. The composition of the crop showed the same abnormalities which are found in the potassium deficient crop.

3. The possibility of utilising the potassium content of straw was examined. In presence of acriflavine the straw proved to be highly beneficial and produced yields which were only slightly below those produced by manuring with potassium sulphate. The same effect was achieved by heating the soil prior to the application of straw. The addition of straw ash was beneficial though the yield was not very high. The decomposition of straw in the waterlogged soil proved to be beneficial after about 3 months. Shorter periods of decomposition led to progressively lower yields. Addition of straw at transplanting depressed the yield below that of the untreated soil. Increases in yield were accompanied by increased percentages of potash in the crop and a lowering of the nitrogen and phosphoric acid contents. Decomposition of straw in waterlogged soil seemed to be incomplete even after four months. Drying and aeration of the straw-treated soil led to further increase in yield.

4. Partial sterilisation of Patheingyi soil led to a crop of normal composition though the yield was only moderate. Compared to this treatment acriflavine, added to the unheated soil, did not produce any benefit to the crop in yield or composition.

5. Analysis of field samples of *amyitpo*-affected and healthy rice plants showed the same contrasts as between potassium-deficient and normal plants. In all cases the diseased plants contained much less potassium but more nitrogen than the healthy plants. Morphological symptoms too were identical with those shown by potassium-deficient plants.

6. Studies in water culture showed that *amyitpo*-affected plants would recover in presence of all the nutrients or with the omission of nitrogen or phosphoric acid though the plants died in nutrient solutions lacking in potassium. Even the healthy plants acquired the characteristics of *amyitpo* disease when grown under potassium deficiency.

7. Field demonstrations were carried out in small plots by application of potassium sulphate soon after the diseased condition was noticed. The plants recovered in every case and became normal in appearance and size.

8. The effect of straw is discussed in relation to the large development of micro-organisms induced by it.

9. The methods of using straw as a source of potash are discussed. The recommendation is made that these are not practicable for field use and that the best procedure for restoring the fertility of Patheingyi soil is to burn the stubble and apply potassium sulphate where required.

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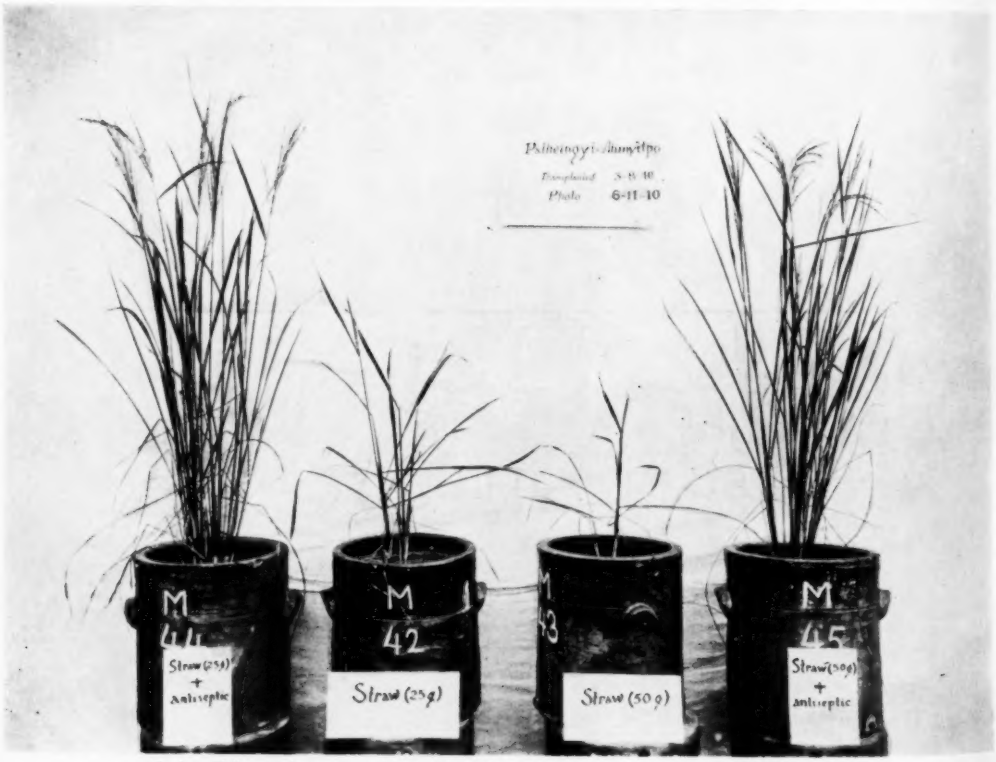
a. Typical amyitpo-affected area in Patheingyi soil.

b. Response of rice to nutrients in Patheingyi soil.

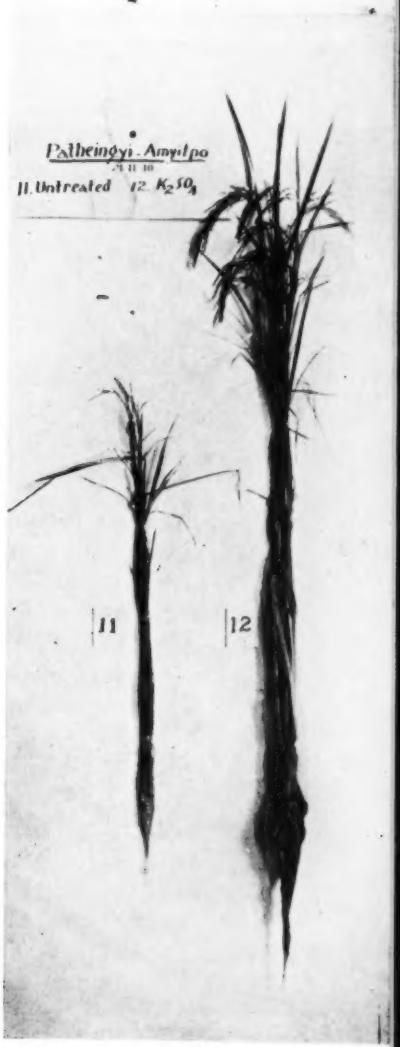
a

b



a. & b. Effect of straw with and without antiseptic on rice in Patheingyi soil.

a



b

a. Typical field samples of rice from *amyitpo* area.

b. Field response of *amyitpo*-affected rice to potassium sulphate in Patheingyi soil.

EXTERNAL CHARACTERS OF THREE FŒTUSES OF THE INDIAN ELEPHANT

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The authors have in their custody three fœtuses of the Indian elephant, *Elephas indicus*, at different stages of development, whose great value lies in their rarity. In so far as we are able to trace references, we find that to date only six fœtuses of the Indian elephant have been remarked on or described in scientific literature (Gray, 1868; Eales, 1925; Hill, 1938; Osborn, 1936, 1942). Hence it is considered that observation on these three fœtuses would be an additional contribution to the meagre knowledge of the developmental anatomy of this species.

Material

Of the fœtuses, the smallest, of 7·7 cm. crown-rump length, hereafter called Fœtus A, has been lying preserved in methylated spirits for many years in the reserve collections of the Government Museum, Madras. It is now on loan for study at the Department of Anatomy, Medical College, Madras. The circumstances in which it was procured are not known. The second fœtus of 13·3 cm. crown-rump length, Fœtus B, has been preserved in methylated spirits for the last nine years in the Anatomy Department of the Madras Veterinary College. This specimen was aborted by a cow elephant under domestication in 1939 at Calicut. The third specimen of 25 cm. crown-rump length, Fœtus C, has been preserved in 5 per cent. formalin in the Anatomy Department of the Madras Veterinary College. It was procured last year at the post-mortem examination of the mother that died in the trench during capture. It was immediately preserved in formalin and later sent over here.

For want of proper facilities on the spot, injection of embalming fluid by the umbilical vessels was not done originally for any of the specimens. The specimens therefore are not ideally preserved, but are well hardened without decomposition. Form and shape are retained and the tissues are reasonably tough. Slight injury in places has been caused by certain extraneous factors. Fœtus A has undergone some damage to the head and soles of all the four feet due to the fixing of pins while mounting the specimen for display. Fœtus B has undergone slight damage to the head; and its abdominal and thoracic organs have been partly eviscerated through a longitudunal incision of the abdominal wall during preservation. Fœtus C is reasonably well preserved except for slight compression of one side due to its lie in the jar containing the preservative solution.

Table I gives a statement of available information regarding comparative size and age of the six Indian elephant fœtusses remarked upon by previous

TABLE I

Comparative Size of Fœtuses of Indian Elephant

(Blanks indicate lack of data)

Serial No.	Author	Year	Place where specimen is preserved	Size C. R. Length in cm.	Age
1	Zimmerman ..	1783	Brunswik Museum	34·0	..
2	Owen ..	1857	R. C. S. Museum	..	6 months
3	Turner ..	1866	..	..	11 months
4	Gray ..	1868	British Museum	6·8	..
5	Toldt (Jr.) ..	1913	..	..	11 months
6	Hill ..	1938	Colombo Museum	31·5	Passed midterm
7	Present authors..	This study	Madras Museum	7·7	..
8	Do ..	do	Anatomy Museum Madras Veterinary College	13·3	..
9	Do ..	do	do	25·0	..

authors and the three specimens included in this study. It is seen that the three fœtuses described here, bridge a gap of size and naturally of age, in which observations on specimens have not been previously made; they form a well graded intermediate series between the specimens in the British Museum and Colombo Museum. A statement of weights and various linear measurements of the three fœtuses is given in Table II.

TABLE II

Statement of Weights and Measurements

Particulars	Fœtus A	Fœtus B	Fœtus C
Weight (after preservation) ..	34 gm.	149 gm.	2154 gm.
	cm.	cm.	cm.
Height, standing ..	5·7	9·2	22·5
Length from tip of proboscis to tip of tail over curve of back	19·3	31·5	68·5
Length from forehead to root of tail, in a straight line (crown rump length)	7·7	13·3	25·0
Length from forehead to root of tail over curve of back	11·6	18·0	36·3
Probocis length, from tip of proboscis to angle of mouth, along curve	2·0	4·5	15·2
Tail length ..	3·0	5·2	11·0
Eye, Palpebral slit ..	0·3	0·5	0·9
Eye bulge ..	0 7×0·7	1·3×1·1	2·2×1·8
Ear pinna ..	1·1×0·6	2·0×1·8	5·0×5·5
Distance from angle of mouth to ear opening ..	2·1	3·0	6·6
Forelimb, from free portion above the elbow to the sole along the limb flexures	3·4	5·3	14·0
Hindlimb, from external angle of ilium to stifle ..	3·3	4·8	10·0
Elbow to nail along flexures ..	2·0	3·6	8·0
Stifle to nail, along the flexures ..	2·0	4·3	9·2
Circumference of forefoot (sole) ..	2·5	4·0	10·0
Circumference of hindfoot (sole) ..	2·5	3·7	10·4
Circumference of body at umbilicus ..	11·5	15·5	41·0
Circumference of the neck ..	7·0	11·9	27·0
Circumference of temporal gland ..	1·2	2·9	6·6
Diameter of temporal gland ..	0·4	0·9	2·2

GENERAL DESCRIPTION OF THE FŒTUSES

Fœtus A (7·7 cm., male)

Head.—All the features of the fœtus are well formed giving it the characteristic appearance of the animal (Fig. 1). The frontal bosses are not marked. The neck is distinct. The ears are small and triangular. The probocis is small and extends down to the level of the carpus. The posterior aspect of the probocis shows only the median longitudinal sulcus. The finger or prehensile lobe at the anterior margin of the lower end of the proboscis has not yet formed. The eyes are small and the lids closed. The palpebral fissure is a narrow open slit indicating the unfused condition of the eyelids. Between the eye and ear, a flattened circular swelling of 4 mm. diameter marks the site of the temporal gland. The upper lip-base of the proboscis—shows an elliptical opening on either side indicating the site of eruption of the future milk tusk. The lower lip is elongated with its tip pointed. Its upper aspect is grooved to accommodate the tip of the tongue. The labial cushions of the upper lip (Eales, 1925) are not distinct.

Trunk (Body).—The body is short and globular. The external genetalia are well formed and show indubitable male character. The penis and its prepuce are well developed. The external orifice of the urethra is a small vertical slit.

Tail.—The tail is short and tapering. It does not show any termina tuft of hairs.

Limbs.—The limbs are well developed but are slender and lack the characteristic stumpy shape found in older fœtuses and the adult. The sole of the fore foot is nearly circular and bears five white, soft nails at the periphery. The nails of the fore foot are in descending order of size, 3, 4, 2, 5, 1; and the third nail has a transverse measurement of 4 mm. The sole of the hind foot is elongated with the heel pointed, and bears four white soft nails. The size of the nails in descending order is 2, 3, 1,4; and the second has a transverse measurement of 2·7 mm. The relation between the circumference of the sole of fore foot and the shoulder height is 1 : 2·3. This point is discussed subsequently.

Skin.—The skin is not pigmented and does not show any hairs. The eyebrows and eyelashes have not begun to erupt. No hair follicles are noticeable even on close observation on the proboscis or on the terminal part of the tail.

Temporal Gland.—A flattened circular swelling of 4 mm. diameter between the eye and the ear marks the site of this gland. Due to slight damage the opening of the duct on the surface could not be made out with certainty.

Fœtus B (13·3 *cm., male*)

Head.—All the features of the fœtus are well formed (Fig. 2). The neck is distinct. The ears are small. The proboscis is small extending only slightly below the level of the carpus. The posterior aspect of the proboscis shows only the median longitudinal sulcus. The anterior margin of the lower end shows the prehensile lobe or 'finger' just developing. The eyes are small and closed and the slit like palpebral fissure indicates the unfused condition of the eye lids. The temporal gland is represented by a flattened circular swelling between the eye and ear. The upper lip-base of the proboscis—shows on either side an elongated opening, the site of eruption of the future milk tusks, and immediately behind this an ill-defined labial cushion. The lower lip is elongated with its tip pointed and its upper aspect grooved to accomodate the tongue.

Trunk (Body).—The trunk is relatively long. Due to the prior evisceration of the abdomimal and thoracic contents it presents flatness of sides and floor,

not showing its real contour as in other specimens. The external genitalia show the penis and prepuce to be well formed; and the external urethral orifice is a small vertical slit. Two small roughened spots in the pectoral region indicate the position of the mammary glands.

Tail.—The tail is tapering and has not developed the terminal tuft of hair.

Limbs.—The limbs are well developed. The sole of the fore foot is nearly circular and bears at its periphery five soft nails. The nails in order of size are 3, 4, 2, 5,1 and the third one measures 5 mm. transversely. The sole of the hind foot is an oval with the heel pointed. The hind foot bears four soft nails in descending order of size, 2, 3, 1, 4. The second nail is 4·5 mm. wide. The ratio between the circumference of the sole of fore foot and the height of the specimen is 1 : 2·3.

Skin.—The skin is not pigmented and does not show hairs in any part of the body.

Temporal Gland.—The gland is 9 mm. in diameter. The opening of the duct is seen on the surface of the flattened swelling formed by the gland, in the postero-inferior quadrant, eccentrically placed 1 mm. above its lower border.

Fœtus C (25·0 *cm., male*)

Head.—The head is slightly compressed. The ears are large. The proboscis is well developed, extending considerably below the level of the forefoot, thus approximating the adult stage. The neck is apparently thick possibly due to its ventral flexure in accord with the marked curve of the fœtus (Fig. 3). The posterior aspect of the proboscis shows three longitudinal sulci, one median and two lateral, which are more distinct in the lower part (Fig. 4). Besides, there are transverse sulci on both the aspects of the proboscis as seen in the adult (Figs. 4 & 5). The dorsal margin of the lower end of the proboscis shows the well developed prehensile lobe or 'finger'. The eyes are small and closed but the eyelids are not fused. Posterior to the eye a flattened circular swelling of 22 mm. diameter marks the temporal gland. The upper lip-base of the proboscis-shows on either side in front of the well defined labial cushion, a deep oval fossa, the site of eruption of the milk tusks. The lower lip is elongated with its tip pointed; its upper face is grooved to accommodate the tip of the tongue.

Trunk (Body) .—The trunk is strongly arched in its dorso-lumbar part indicating the postural adaptation of the developing fœtus in the uterus. The external genitalia are well formed. The external urethral orifice is 'Y' shaped with the two dorsal diverging limbs shorter as in the adult. The

prepuce nearly completely covers the penis, exposing the tip of the glands at the preputial orifice. The two pectoral mammæ are well developed with the nipples seen as small conical elevations, placed 2 cm. apart.

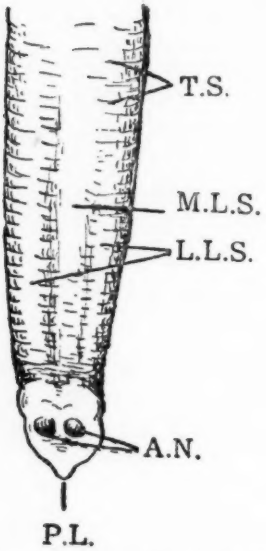

Fig. 4. Proboscis of Fœtus C, showing sulci on the ventral aspect. *A.N.*, anterior nares; *L.L.S.*, lateral longitudinal sulcus; *M.L.S.*, median longitudinal sulcus; *P.L.*, prehensile lobe or 'finger' at the tip of the proboscis; *T.S.*, transverse sulci.

Fig. 5. Proboscis of Foetus C, dorsolateral view, showing *P.L.*, prehensile lobe; *T.S.*, transverse sulci.

Tail.—The tail is proportionately longer and lacks a terminal tuft.

Limbs.—The limbs are well formed. The sole of the forefoot is oval in outline with the broader end posterioly (Fig. 6) and is flesh coloured. It bears five nails of the same colour. The nails are slightly hard indicating the degree of keratinisation. Their size in decreasing order is 3, 4, 2, 5, 1, the third measuring 12 mm. transversely. The sole of the hind foot is an elongated oval in outline with the narrow end posteriorly and is flesh coloured. It bears four nails of the same colour which are partly keratinised as in fore foot. Their size in decreasing order is 2, 3, 1, 4, the second measuring 11 mm. in width. The relation between the circumference of the forefoot and the height is 1 : 2·25.

Skin.—The skin shows a generalised pigmentation throughout the external body surface except the soles of the feet. The degree of pigmentation,

however, varies in different parts of the body. The skin on the dorsum of the body, crown of the head, and the anterior aspect of the proboscis in its upper half is dark brown; that on the ventral aspect of the trunk (body), limbs and the lower part of the proboscis is light brown. The difference in the degree of pigmentation of the skin is due to the density of distribution

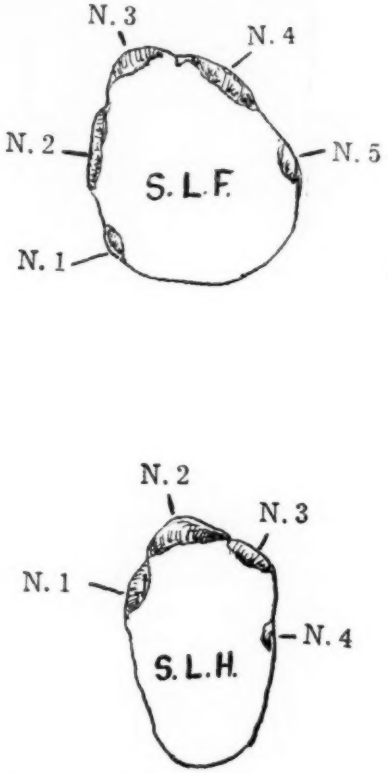

Fig. 6. Outline of sole of fore foot and that of hind foot of Fœtus C. *S.L.F.*, role of left fore foot; *S.L.H.*, sole of left hind foot; N. 1, N. 2, etc., indicate nails of corresponding digits.

of the chromatophores. A thin layer of the epidermis was dehydrated, cleared and mounted in Xylol damar and examined under the microscope (microphotograph Fig. 7). It shows the squamous cells with the chrotomatophores arranged in their cytoplasm with varying distribution in different cells. The intercellular cement substance also shows chromatophores of finer size.

The skin all over the body shows numerous macroscopic pits, the sites of invagination of the epidermis to form the hair follicles. The hair follicles on the skin of the dorsal aspect of the proboscis are represented in Fig. 8.

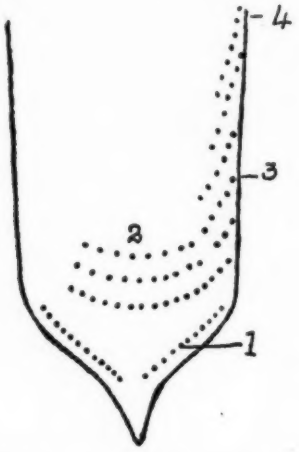

Fig. 8. Hair tracts on the dorsal aspect of the lower part of the proboscis of Fœtus C. 1, 2, 3, 4, are regions showing different directions of hair tracts.

The dorsal face of the tip of the proboscis shows white conical hairs sprouted above the epidermal surface. In no other part of the body (including the eyebrows, eyelids, and the tip of the tail) were the sprouting of the hairs on the epidermal surface observable. Hill (1938) mentions that the skin surface is everywhere hairless in the larger fœtus (31·5 cm. C.R. length) described by him.

Histology of the skin.—Vertical sections of the skin reveal under the microscope five to six layers of wavy stratified squamous epithelium constituting the epidermis which is moulded on the dermis (Fig. 9). The deepest layer of the epidermis consists of columnar cells and above them there are three to four layers of squamous cells, having affinity for the basic stain. The superficial layer consists of a narrow zone of transversely elongated scales keratinised to form the stratum corneum having affinity for the acid stain. At several places the epidermis invaginates into the dermis forming the rudiments of hair follicles. The hair follicles are still primitive not exhibiting the forked bottom embracing the vascular dormal papilla. The cells of the hair follicle are all protoplasmic, not showing keratinisation of the central cells to form the hair.

The dermis consists of a loose network of fibroblasts which preponderate over fibres that are just being formed around the rudiments of the hair follicles. The upper part of the dermis is papillated adapting itself to the wavy epidermis.

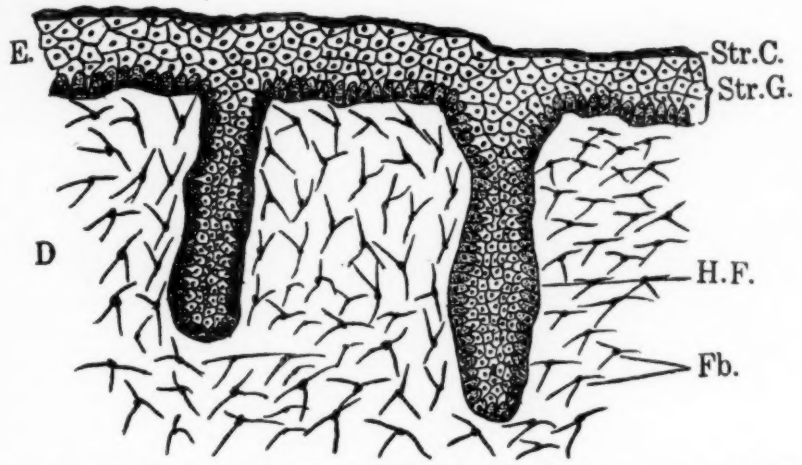

Fig. 9. Drawing of vertical section of the skin of Fœtus C. *D*, dermis; *E*, epidermis; ***Fb.*** fibroblasts; *H.F.*, developing hair follicle; ***Str. C.***, stratum corneum; ***Str. G.***, stratum germinativum.

Sebaceous and sweat glands are not yet developed and consequently arrectores pilorum muscles are not differentiated.

Mammary gland.—The nipples are situated one on each side at a distance of 10 mm. from the median line in the pectoral region. Histological examination of the mammary gland reveals a close net-work of fibroblasts in which are ducts lined by stratified epithelium of three to four layers of short columnar cells constituting the undifferentiated stage of the gland (Fig. 10).

Temporal gland.—Posterior to the eye a flattened circular swelling of 22 mm. diameter marks the temporal gland. The opening of its duct on the surface of the skin covering the gland is in the postero-inferior quadrant, eccentrically placed 6 mm. above its inferior border.

Histology of the temporal gland.—A tangential section of the gland (Fig. 11) across the main duct reveals several lobules separated by an abundant interlobular connective tissue in which the main duct is running to the surface of the gland receiving several small lobular ducts all round. The main duct is lined by stratified squamous epithelium thrown into folds

that occlude the lumen. The deepest layer of the epithelium consists of columnar cells, and the other layers, of squamous cells. The wall of the duct is fibro-muscular consisting of a thin layer of plain muscle fibres next to

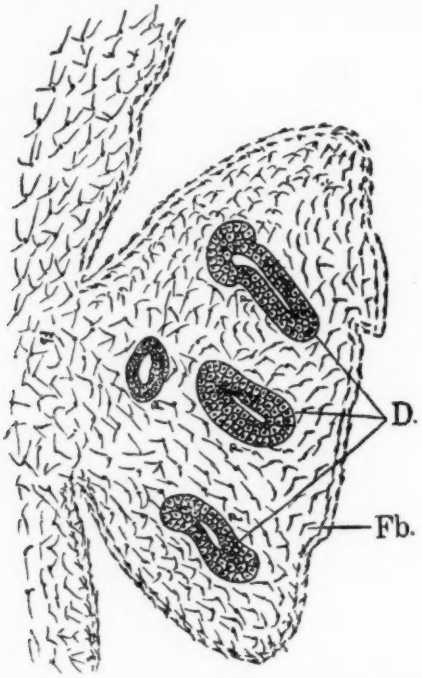

Fig. 10. Drawing of vertical section through the nipple of Fœtus C, showing the rudiment of the mammary gland. *D*, developing lactiferous ducts (tubules); *Fb.*, fibroblasts.

the epithelium and a thick outer layer of connective tissue. The lobula ducts are small and are lined by a double layered epithelium of short columnar cells.

The lobules show several irregularly tubular alveoli that are seperated by a small amount of interalveolar connective tissue. The alveoli have a thin wall of connective tissue lined by a double layered epithelium of short columnar cells.

From the structure and staining affinity of the alveolar epithelium the authors conclude that the temporal gland of the elephant is a modified sweat gland of the tubulo-alveolar type and agree with Eggeling (cited by Eales, 1925). We have personally observed in the adult animal the exuding secretion of the temporal gland to be a viscid dark fluid during ' must ' which is generally

considered to be a period of sexual excitement. The term 'oily' has been applied by some authors (Evans, 1910) to describe this secretion. It appears that the term has been mistakenly used to denote the viscous nature of the secretion. The question suggests itself, whether the gland is apocrine in

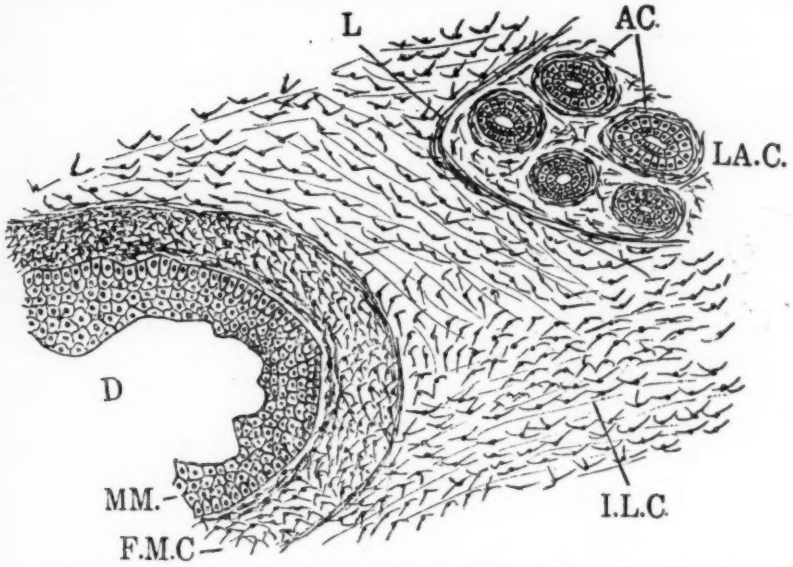

Fig. 11. Drawing of tangential section of the Temporal gland of Fœtus C. *AC.*, Acini; *D.*, main duct; *F.M.C.*, fibro-muscular coat; *I.A.C.*, interaciner connective tissue *I.L.C.*, interlobular connective tissue; *L.*, lobule of the gland; *M.M.*, mucous membrane of the duct.

nature containing in its viscid secretion ingredients of partial cellular disintegration? This can only be determined by a study of the temporal gland at the time of 'must'.

Discussion of certain Developmental Features

Auricula.—The illustrations in Fig. 12 show the auricula in the three fœtuses drawn to natural size under the camera lucida.

The auricula in fœtus A is a triangular flap with its anterior border (base) attached round the external auditory meatus. The superior border is short and nearly horizontal. The posterior border is longer than the others and is oblique, directed downwards and forwards. The inferior angle shows anticipations of the appearance of the inferior border.

The auricula in fœtus B is quadrilateral with the anterior border attached round the external auditory meatus. The superior border is markedly convex and is longer than the other borders. The posterior border is nearly as long as the superior, directed with an increased obliquity downwards and forwards. The inferior border is nearly half as long as the posterior with its concavity directed downwards.

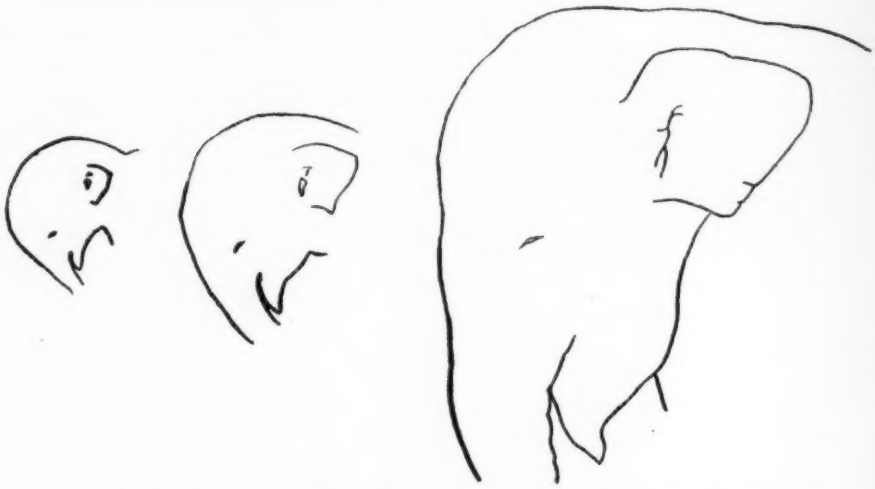

Fig. 12. Camera lucida drawings of heads of fœtuses A, B and C, to show relative growth of auriculæ; originally drawn to natural size and proportionately reduced in reproduction.

The auricula in Fœtus C is pentagonal and leaf-shaped, with an anterior border attached round the external auditory meatus. The superior border is the longest and represents two adjacent sides of the pentagon, consisting of an anterior oblique part above and in front of the external auditory meatus, and a posterior horizontal part. The posterior border is shorter than the superior with more increased obliquity downwards and forwards. The two small linear folds near its inferior part seen in the Fig. are not permanent but due to folding of the edge. The inferior border is shorter than the posterior, with its concavity directed downwards. The combined inferior and posterior borders are symmetrical with the superior border.

For comparing the proportionate growths of the auriculæ in the three fœtuses, the following technique was devised. The temporal region between the posterior angle of the eye and the external auditory meatus was taken as a relatively stable region for comparing the axial lengths of the auriculæ

measured from the external auditory meatus to the apices. A ratio was worked out with the auricular length as the numerator and the temporal length as the denominator. This ratio in the three fœtuses A, B, and C is 8:15, 20:21 and 47:44, respectively. The above indicates that there has been much accelerated growth of the auricula as compared with the rate of growth of the temporal region during development from stage A to C.

The auriculæ of the adult African elephant are very large and foliate, extending far above the level of the crown so that both together form a hood to cover the crown enabling the animal to withstand the heat of the tropics in the open. This is in marked contrast to the comparatively small ears of the Indian elephant which is known to be unsuited for an open life in the tropics and prefers a natural habitat in thick jungle. Fig. 13 which is a tracing from authenticated photographs (Evans, 1910) illustrates the contrasted auriculæ.

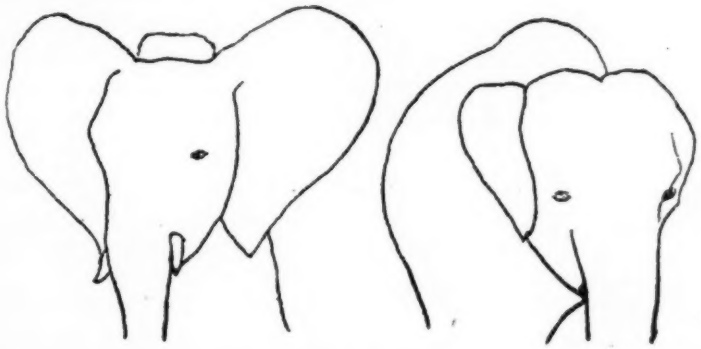

Fig. 13. Tracings from photograph of the adult African elephant and Indian elephant to show the difference in form of the auriculæ.

The evidence from fœtal anatomy indicates that a large auricula is not to be considered as a primitive condition but as a specialization. The African elephant illustrates this tendency in an exaggerated manner.

Proboscis.—Recognising the fact that proboscis has been evolved progressively in the evolution of the Proboscidea, we wished to make a comparison of the relative lengths of the proboscis of the three fœtuses with that of the adult. With this in mind we also measured the length of the proboscis and the height at shoulder of an adult tusker in the Madras Zoo. Due to the constant active mobility of the proboscis very accurate measurement of the same, it must be admitted, was not possible. Approximately the ratio of the length of proboscis to height at shoulder is 1 : 1·5, in the tusker.

The corresponding ratio of proboscis length to height at shoulder in the fœtuses was 1:2·8 in Fœtus A; 1:2 in Fœtus B; and 1:1·5 in Fœtus C. Thus it is evident that Fœtus C has attained the relative length of the proboscis as in the adult. There is a notion in general that the new-born elephant calf has a shorter proboscis to facilitate mammary sucking. But from the observation on Fœtus C, this view seems to be unwarranted.

Neck.—Eales (1925) remarks that the neck in the African elephant fœtus is relatively longer and more slender than in the full grown. The neck in Fœtus A and Fœtus B is distinct and slender. But in Fœtus C the neck is not so distinct. To make a comparison between the relative size of neck to body, we adopted the following procedure. The ratio of the circumference of the neck to the girth at the umbilicus was worked out in the fœtuses and also in two adult elephants in Madras Zoo. The ratio is 1:1·7 in Fœtus A; 1:1·5 in Fœtus C; and 1:1·9 in the adults. The ratio in Fœtus B has not been taken as its abdomen was already eviscerated and hence the girth at umbilicus was not reliable. This ratio is found approximately equal in Fœtus A (the smallest fœtus) and the adult. But Fœtus C shows the ratio altered in favour of the neck. This we consider as due to the uterine adaptation of the fœtus involving the ventral flexure of the neck giving it an apparently stouter neck.

Hair tracts.—There is no evidence of any eruption of hair anywhere on the body in Fœtuses A and B. In Fœtus C, as already noted, there is generalized development of hair follicles all over the body. But sprouted hairs are seen only in the lower part of the dorsal aspect of the proboscis. The hair tracts in this region are represented in Fig. 8. Though hairs were not noticed in all the follicles due to partial peeling of the epidermis, the pattern of the hair tracts here could be studied. The hair tracts along the sides of the dorsal aspect of the proboscis were oblique rows (marked 3 in Fig. 8). These oblique rows tend to become more transverse in the lowest part of the proboscis. The obliquity of these tracts tend to become nearly vertical as they are traced upwards (egion marked 4 in Fig. 8). On either side of the edge above the terminal finger of the proboscis in the region marked 1, the hair tracts are pronouncedly oblique and meet each other over the base of the finger. A little above this region are some transverse tracts of hair marked 2 in the Fig. The hairs seen were white and conical and the general direction of individual hairs was downwards and medially.

External Genitalia.—All the three fœtuses under study here happen to be males. This is a misfortune for the authors that they could not study the female genitalia. But it has made possible the observation of the progressive development of the male genitalia.

The external urethral orifice.—In Fœtus A it is a small vertical slit (Fig. 14) whose ventral end is very acutely pointed and on close examination under a hand lens is found to be in continauation with the recently closed lips of the urethral groove. This is illustrated in the inset diagram by the side

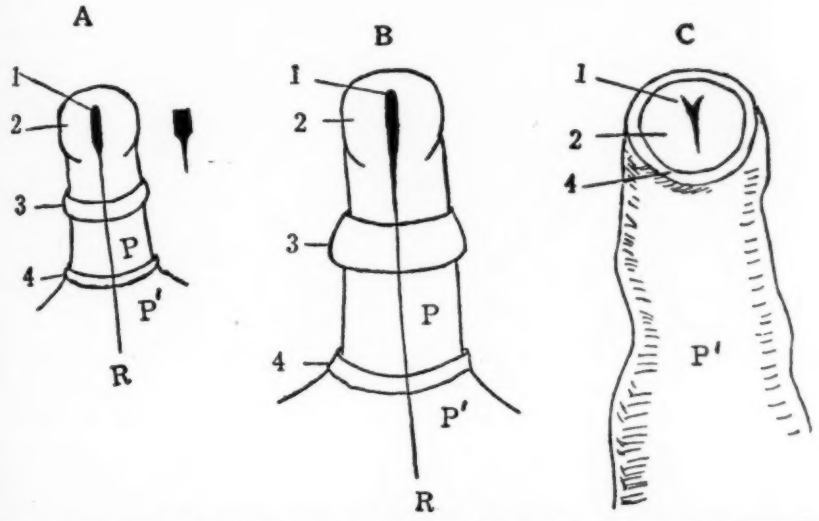

Fig. 14. Drawings of the external genitalia of Fœtuses A, B and C. (1) external urethra orifice (the inset by the side of A is a magnified drawing of the ventral part of 1 to show the closing urethral groove); (2) glans penis; (3) preputial ring; (4) preputial orifice; *P*, internal layer of prepuce; *P'*, external layer of prepuce; *R*., raphe.

of the Fig. showing the condition in Fœtus A. In Fœtus B also, a similar condition is noted. In Fœtus C the external urethral orifice is 'Y' shaped with the two dorsal diverging limbs shorter. This is similar to the condition in the adult.

Penis.—In Fœtuses A and B the exposed part of the penis is cylindrical surmounted by a bulbous glans. The glans shows on its ventral aspect the external urethral orifice. The penis behind the glans is only partly covered by the internal layer of the prepuce with the preputial ring a little below the external urethral orifice. The preputial orifice is proximal to the preputial ring and at the orifice the external layer of the prepuce is continuous with the skin of the abdomen. The preputial raphe is clearly seen. The glans is more bulbous in Fœtus B than in Fœtus A. The preputial ring is also better marked in Fœtus B. In Fœtus C the penis is nearly completely covered by the prepuce exposing only the tip of the glans at the preputial orifice, as in the quiescent state in the adult (Fig. 15).

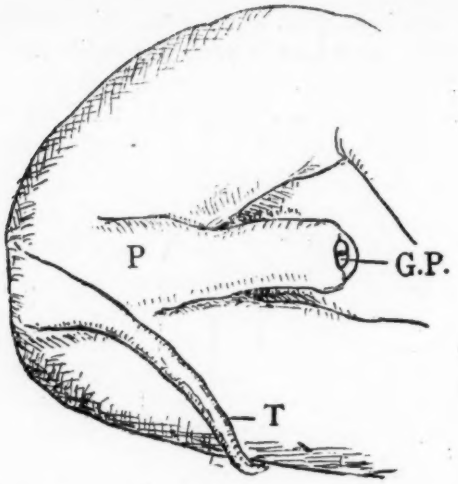

Fig. 15. Drawing to show the external genitalia of Fœtus C. *G.P.*, glans penis; *P*, prepuce; *T.*, tail.

Relation between the circumference of the forefoot and the height at shoulder. —The relation between the circumference of the forefoot and the height at the shoulder is regarded as constant in the adult. The ratio is 1:2, well known to elephant hunters who judge the size of the animals being tracked by the foot prints. This ratio is 1:2·3 in Fœtus A; 1:2·3 in Fœtus B; and 1:2·25 in Fœtus C. The ratio which is less than 1:2 in the fœtus probably becomes gradually changed after birth due to the spreading of the sole with bearing of the weight.

ACKNOWLEDGEMENTS

The authors wish to express their extreme gratitude to Dr. A. Aiyappan, Superintendent of the Madras, Museum for placing Fœtus A at their disposal for study, and to the late Dr. Devadoss, Calicut for presenting Fœtus B to the Anatomy Department of the Madras Veterinary College, and to Dr. P. G. Rozario, Inspector of Live-stock, Calicut, for sending Fœtus C to the Anatomy Department of the Madras Veterinary College. They also wish to express their thankfulness to Dr. S. V. Mudaliar, G. M. V. C. Principal, Madras Veterinary College for facilities given for this work and Dr. K. S. Nair, G. B. V. C., M. R. C. V. S., D. T. V. M., Director of Animal Husbandry Department, Madras, for his continued and kindly interest in this research.

SUMMARY

The external characters of three fœtuses of the Indian elephant, ***Elephas indicus***, of 7·7 cm., 13·3 cm., and 25·0 cm. C. R. length have been figured

Fig. 1. Photograph of Fœtus A of 7·7 cm. C. R. length.

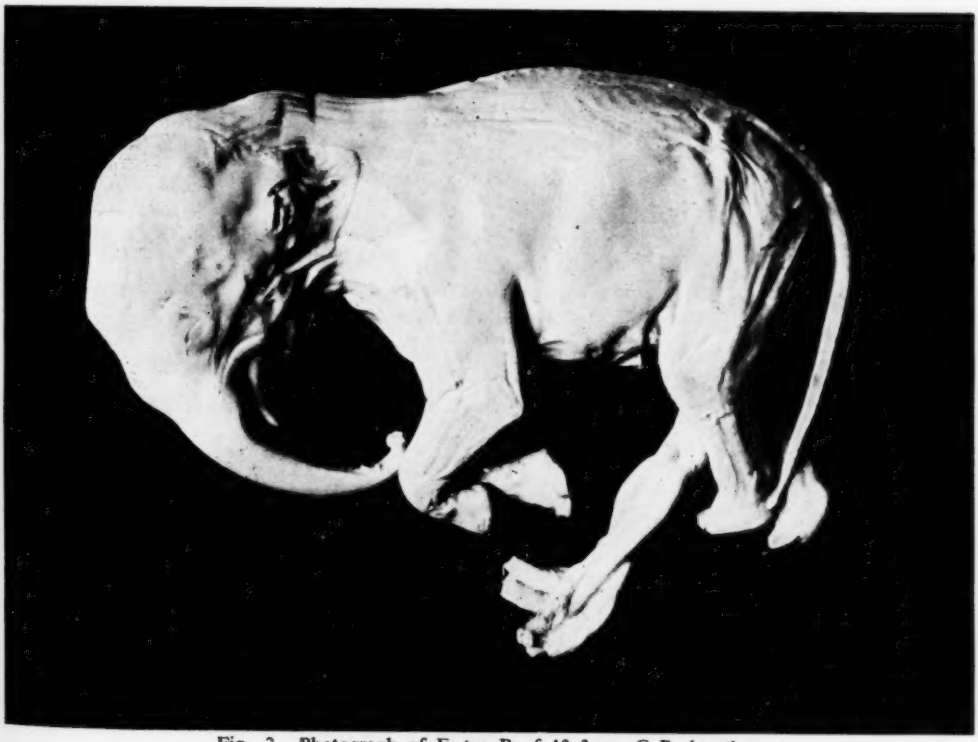

Fig. 2. Photograph of Fœtus B of 13·3 cm. C. R. length.

Fig. 3. Photograph of Fœtus C of 25·0 cm. C. R. length.

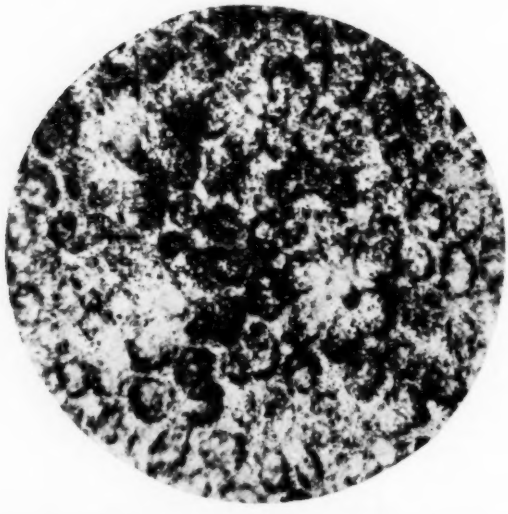

Fig. 7. Microphotograph of epidermis of Fœtus C to show chromatophores.

and described. In size these fœtuses form a valuable intermediate series between fœtal specimens previously noticed in scientific literature. Stages in the development of the auricula from a small triangular flap to a shape approximating the adult condition, are seen. The auricula shows a relatively accelerated rate of growth than the temporal region. The relation between the neck and umbilical girth has been studied. In the two smaller fœtuses (7·7 cm. and 13·3 cm.) the skin is not pigmented and it is devoid of hair follicles. In the largest fœtus of 25 cm. there is generalized pigmentation of skin with varying intensity in different regions; and there is over the surface, development of hair follicles, which are mostly primitive and do not show keratinization of the central cells to form hairs. Erupted hairs are found only on the lower part of the dorsum of the proboscis. Here the general pattern of the hair tracts is oblique as traced downwards and becoming vertical as traced upwards. A little above the 'finger' of the proboscis the tracts are transverse. The direction of hair on probosis is downwards and medially. The temporal gland has been studied in detail in the 25 cm. fœtus. Its histology shows it to be a modified sweat gland of the tubulo-alveolar type. A question is put forward whether it might be of apocrine nature. The mammary glands are pectoral and are found to be still in an undifferentiated stage even in the largest fœtus. All the three fœtuses are male and show grades of differentiation of the external genitalia. In the earlier fœtuses the closure of the urethral groove could be noted and the largest specimen has features nearly similar to the adult. The ratio of the circumference of the sole of the forefoot to the shoulder height shows a gradual transition, but is different from that in the adult; the adult condition is probably secondary to a functional spread of the sole consequent on weight bearing.

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DEVELOPMENTAL STUDIES

IV. The Origin, Development and Morphology of the *Floiaceous* Stipules in *Pisum sativum* Linn.

By Gopal Chandra Mitra

Received March 7, 1949
(Communicated by Prof. G. P. Majumdar, F.A.SC.)

Introduction

Leaves of the *Leguminosæ* are in general compound and stipulate. Many of them are provided with pulvini which bring about their characteristic movements. In certain of the Pulses the stipules become foliar in nature and function with corresponding reduction in the leaf surface. This reduction in leaf surface is effected by the transformation of the terminal and upper leaflets into tendrils in Pea and of the entire upper leaf into tendrils in *Lathyrus aphaca*. The stipules in these plants are termed *foliaceous*.

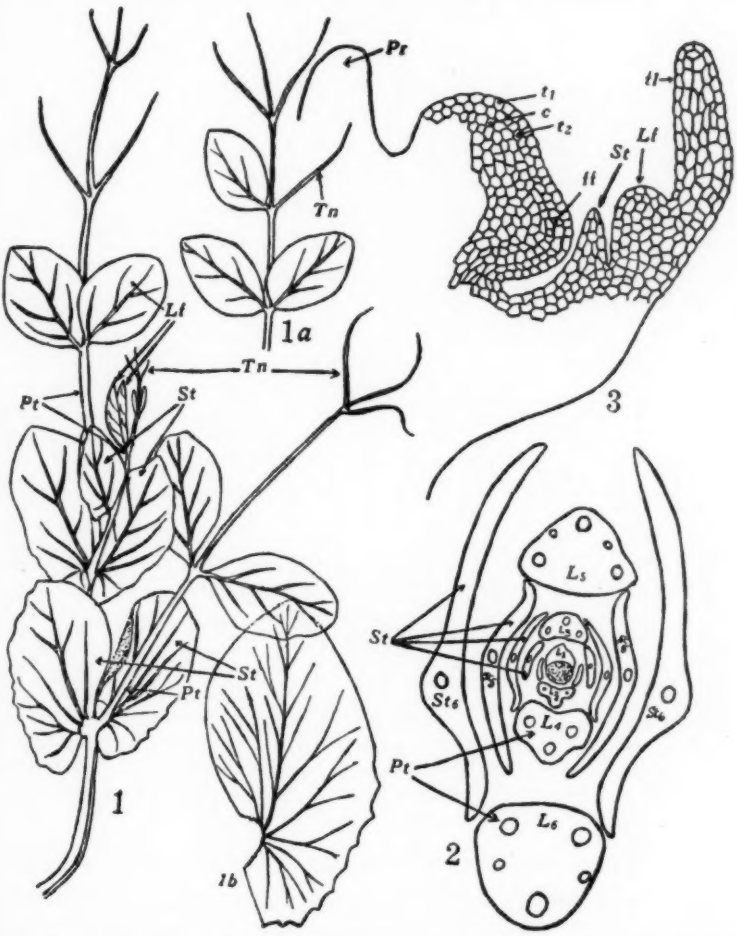
Trécul in 1853 reported that the leaves of most of the *Leguminosæ* fall into the basifugal group. Eichler (1861) supported the observations of Trécul but added further that "In *Pisum sativum* the stipules appear first as lateral structures of the first order". Van Tieghem (1891) for the first time discussed the nature of the tendrils in *Pisum* and concluded that they are equivalent to the terminal leaflet *plus* the distal pair or pairs of leaflets of the compound leaf. Reeve (1948) has recently studied the late embryogeny and histogenesis in *Pisum sativum*. Besides the above few references the author has not found any recent literature on the histogenesis and development of leaves, leaflets, stipules and tendrils of *Pisum sativum*. The origin and development of these structures in this species have been studied for the elucidation of their true morphologies.

The present paper deals only with the origin and development of the *foliaceous* stipules and is a further contribution to the systematic study of the different types of stipules in angiosperms. The methods followed are the same as in previous cases (Mitra, 1945, 1948 *a*, 1948 *b*). All outline figures and those of cellular details have been drawn under camera lucida.

External morphology of the leaves and the stipules

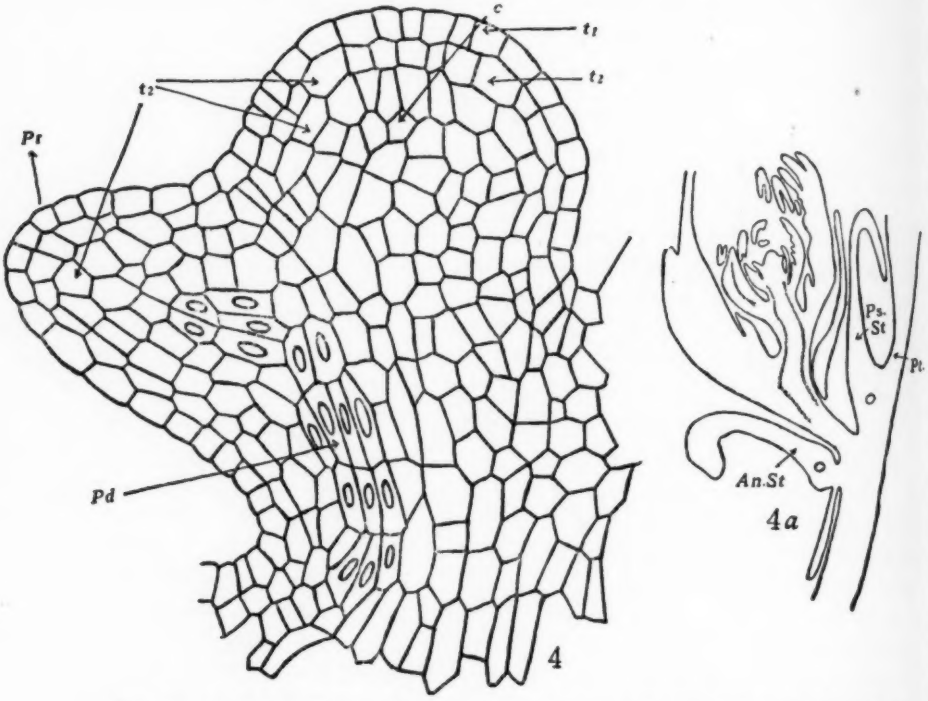
Pisum sativum is a weak annual herb. It is a tendril climber and its stems and petioles are hollow. Leaves are stipulate and pinnately compound with subterminal pair or pairs of leaflets transformed into tendrils

(Figs. 1 and 1 a). The base of the leaf is associated with a pair of *foliaceous* stipules. Figs. 1, 1 *a* and 1 *b* show the external morphology of the stipules, their vascular supply and their relation to the leaves and the axis. Fig. 2



Text-Figs. 1 to 3.—Fig. 1, morphological drawing of a young shoot showing the position and relation of the stipules at nodes (½ Nat. size). Fig. 1 *a*, terminal part of a leaf showing one leaflet and one tendril of a pair (½ Nat. size). Fig. 1 *b*, a detached stipule showing its venation (½ Nat. Size). Fig. 2, a transection of a shoot apex showing arrangement of leaves and their associated pairs of stipules (× 33). Fig. 3, a longisection of a shoot apex showing the initiation of a foliar pri-mordium (*ff*), the stipule (*st.*) and the leaflet initials as papillate outgrowths (*Lf.*); t_1 and t_2, outer and inner layer of tunica; *C*, corpus and *Pr.*, Primordium; and *tl.*, the terminal region of the Primordium (× 186).

is a transection of a shoot apex showing the alternate and distichous arrangement of the leaves and their relation to the associated stipules.



Text-Figs. 4 and 4 *a*.—Fig. 4, a longisection of a shoot apex showing anticlinal and periclinal divisions in t_1 and t_2 and of the corpus (*c*.), the development of a primordium (*Pr*.) and its prodesmogen strand (*Pd*.) from the outer layer of *c*. (× 500). Fig. 4 *a*, a longisection of a shoot apex showing the primordia in different stages of development and an adult leaf with a pair of stipules with their relation to the petiole and the node. *Ps*., *St*. and *An. St*., posterior and anterior wings of the stipule (× 16).

Origin and early development of the leaf primordium

Fig. 3 is a longisection of the shoot apex which is asymmetrical due to the initiation and development of the youngest primordium. The free apex is dome-shaped and consists of two layers of tunica (t_1 and t_2) and a corpus (*c*) without any evident zonation (Fig. 4). A comparison with Reeve's (1948) Figs. 10 and 11 (page 596) show the identical nature of cellular organisation at the adult and the epicotyl apices. Reeve's report of 3 to 4 layers of tunica on the adult shoot of *Pisum*, (page 597) is not borne out by our observation. It is true that a third layer of cells internal to t_2

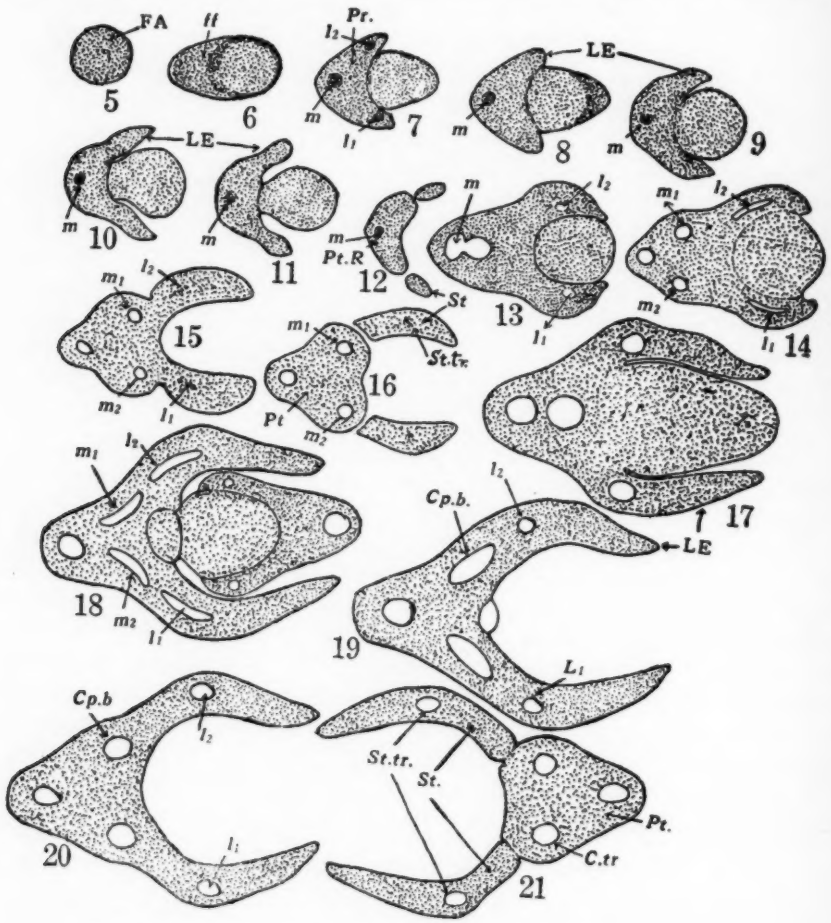
layer is sometimes discernible, but careful examination shows that this layer is derived from the latter by periclinal divisions and is not continuous, like t_1 and t_2, over the apical dome. The corpus is not very well differentiated either in cellular organisation or in its staining capacity. The outer layer of the tunica (t_1) divides exclusively by anticlinal walls and the inner (t_2) by anticlinal and periclinal walls at the time of primordial emergence. The active anticlinal divisions in the outer and the inner layers of tunica in a particular sector of the apical dome bring about transverse extension of the latter in that particular area (Figs. 5 and 6). This is the indication of the initiation of a primordium. The simultaneous periclinal divisions in the inner layer of tunica (t_2) and in the outer cells of the corpus give rise to the volume growth of this particular sector resulting in the formation of a papillate outgrowth (hump) on the surface of the apical dome which later becomes the foliar primordium (free limb) (Figs. 3 and 4 *a*). Exclusive transverse divisions of the corpus derivatives in the sixth internode from the free apex cause the organisation of a late file meristem whose activity brings about quick elongation of the internode.

The outermost layer of cells of the corpus at the region just below the point of emergence of the primordium divide and form the prodesmogen cells (procambial initials of Reeve, page 596) recognised by their staining capacities and longitudinal divisions. They soon become continuous with the acropetally differentiating desmogen strand (procambium strand of Reeve). The primordium is erected under the influence of and followed closely by this strand coming from the axis as its median trace. A little later two more traces from the axis enter the laterally extending base (axial component) of the primordium around the axis. Thus the three traces, one median and two laterals, enter the base of a primordium at different stages of its development. Protoxylem is seen to differentiate for the first time at the base of the free limb and its subsequent differentiation is both acropetal in the elongating primordium and basipetal in the axis.

Origin of the leaflets and the stipules

The primordium, early in its ontogeny, develops papillate outgrowths along its adaxial margins a little behind the terminal region (Figs. 3 and 4 *a*). Periclinal divisions in the subepidermal layer which later extends into underlying cells give rise to these papillate outgrowths which are acropetal in their origin and development. Figs. 7 to 11, 13, 14, 17 and 18 show radial extension of the central region and tangential extension around the axis of the lateral arms of the base. It also extends vertically along with the elongation of the internode with which it is congenitally united, by intercalary

growth. At this stage the axis is seen to consist of a mantle (leaf base, and an axial core. From the axis the base begins to separate at its extreme ends and becomes completely free at some distance upward. The base



Text-Figs. 5 and 6.—Transections showing the free apex and its horizontal extension in the initiation of the foliar primordium (*ff*). (× 186).

Text-Figs. 7 to 12.—Serial transections of the base of primordium 2 from the free apex showing development of the base and the origin of the stipules (*St.*) and the courses of the leaf traces. *Le.*, extending lateral arms; *m.*, median trace; l_1 and l_2, lateral traces (each × 80). Fig. 8 is 16 μ above Fig. 7; Fig. 9 is 16 μ above Fig. 8; Fig. 10 is 16 μ above Fig. 9; Fig. 11 is 8 μ above Fig. 10; and Fig. 12 is 16 μ above Fig. 11.

Text-Figs. 13 to 16.—Serial transections of the base of primordium 3 from the free apex showing development of the base and the origin of the stipules and the courses of the leaf traces

(each × 80). m_1 and m_2 secondary median traces ; *St. tr.*, stipular trace. Fig. 14 is 32 μ above Fig. 13 ; Fig. 15 is 40 μ above Fig. 14 ; and Fig. 16 is 24 μ above Fig. 15.

Text-Figs. 17 to 21.—Serial transections of primordium 4 showing development of the base and the origin of the stipules and the courses of the leaf traces (each × 80). *cp. b.*, composite bundle of the secondary median trace and a branch of the lateral ; *c. tr.*, composite trace in petiole. Fig. 18 is 40 μ above Fig. 17 ; Fig. 19 is 32 μ above Fig. 18 ; Fig. 20 is 32 μ above Fig. 19 and Fig. 21 is 48 μ above Fig. 20.

now being free from the axis grows for a very short distance (not even 2 mm. in the adult leaf) and then resolves into three parts (Figs. 15, 16 and 19 to 21). The central region becomes the petiole and the two extending lateral arms develop directly into the stipules. Thus the gradual separation of the lateral arms or wings while the base is extending vertically in union with the axis and their complete separation into the stipules at a point higher up on the latter cause their oblique insertions on the axis, and the continuation of the free wings directly into the stipules make them appear cauline in their adult morphology. It is thus seen that the stipules have no separate primordia like the leaflets (Figs. 3 and 4 *a*).

The leaf-primordium at the very early stage of development is distinguished into a 'lower leaf' with a pair of stipules just differentiating and an 'upper leaf' with a pair of leaflet primordia with no indication of the petiole in between the two which is its normal position in the adult leaf (Fig. 3).

Figs. 7 to 12 represent serial transections of the base of primordium 2 from the apex. The lateral traces do not extend upto the upper region of the base whose height is 76 μ and the total height of the primordium is 240 μ. The wings of the base at its upper region separate from its central region into the two stipules which continue their free growth for 24 μ. Figs. 13 to 16 represent serial transections of the base of primordium 3 from the free apex. The base is 96 μ high and the height of the primordium is 418 μ. In the base the median trace splits into two; one of these facing the adaxial surface split again into two (secondary median bundles, m_1 and m_2) and they then move obliquely towards the sides and cause the bulging opposite them (Figs. 14 and 15). The laterals on the other hand give off branches into the arms of the base before its separation from the axis and on their way to unite with the branches of the secondary medians (m_1 and m_2). The branches of the laterals stimulate the growth of the free arms into the stipules and become their vascular system. The stipules continue their free upward growth for 128 μ. Figs. 17 to 21 represent serial transections of the base of primordium 4 from the free apex. The base is 152 μ high and the height of the primordium is 1440 μ. In the base the oblique courses of the laterals which are noticed in primordium 3 proceed further and unite with the

secondary median bundles (m_1 and m_2). Later, in older leaves, one or two branches from this united bundles are also given off to the stipules. The stipules with the branches from the above two sources continue their free upward growth for 340 μ.

The petiolar region which is initiated in primordium 3 is seen to increase in length in primordium 4 by intercalary growth. On comparing Figs. 10, 11, 16 and 21 it is seen that the stipules are continuations of the extending lateral arms of the base just after its separation from the axis.

The Table below shows the relative rates of growth of the different parts of the developing primordia:

	Primordium			
	1st	2nd	3rd	4th
Base—From the pt. of insertion on the axis to the level of separation of the stipules	nil	76	96	152
Petiole—From the level of separation of the stipules to the pt. of insertion of first pair of basal leaflet primordia	nil	8	24	144
Internode—Between the 1st and the 2nd pair of leaflet primordia	nil	nil	88	208
Total length of the primordium	76	240	640	1440

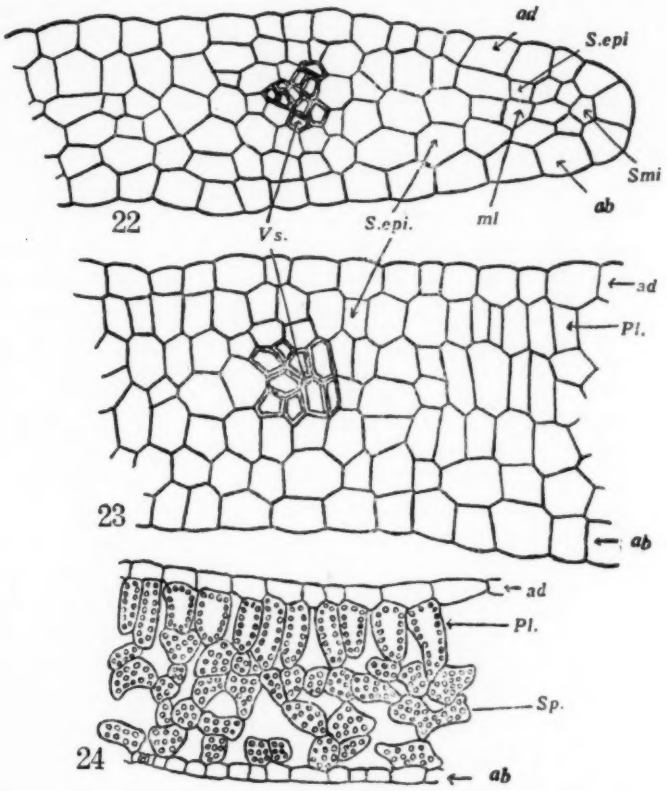
(Measurement expressed in microns)

Of the different parts of the leaf primordium the base is the earliest to start a rapid growth in length. The growth in length of the petiolar region is at first slow but it soon becomes very great. It is noticed that even in primordium 4, the third pair of leaflet primordia have not developed though the length of the primordium is 1440 μ, but they are formed in the same primordium at the apices of the adult plant. The terminal region of the primordium shows very rapid meristematic activity of the subapical cell and its derivatives in the ontogeny of the leaf. The transverse growth of this region is much less when compared to its rapid elongation and as a result the upper region becomes filiform, loses its activity and turns into a tendril. The adult morphology of the leaf also shows the rapid growth in length of the upper region (Figs. 1 and 1 *b*).

Marginal growth and tissue differentiation of the stipules

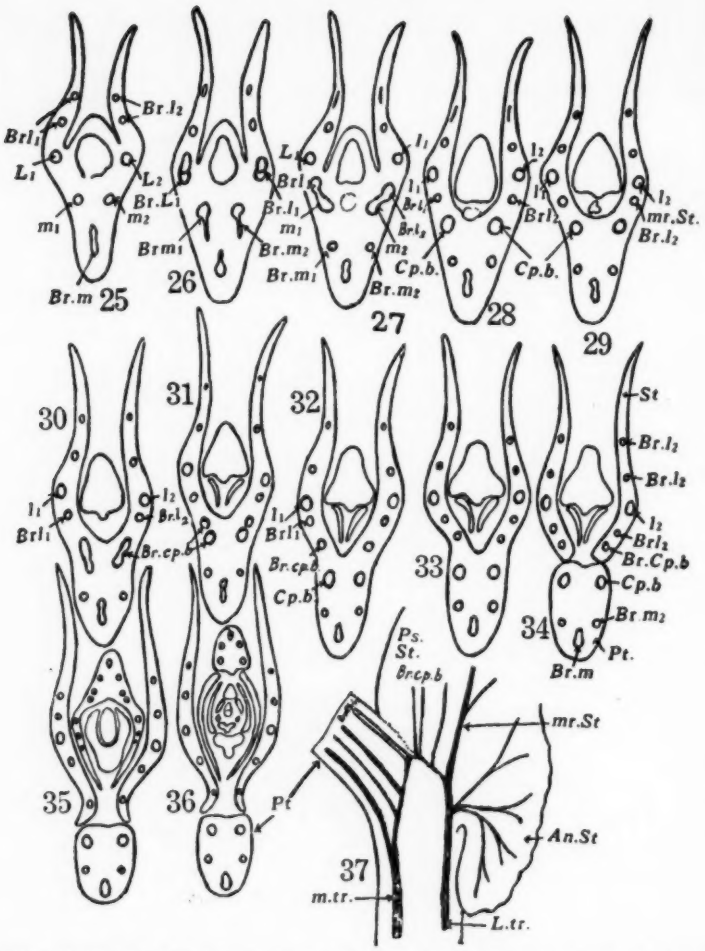
The marginal growth of the stipules takes place by anticlinal divisions of the submarginal initial (Smi) on its two faces (Fig. 22). These divisions

give rise to the adaxial and the abaxial subepidermal cells. By periclinal divisions in the abaxial subepidermal cells the middle layer is derived (Fig. 22). The marginal cells divide exclusively by anticlinal walls forming



Text-Figs. 22 to 24.—Transections of the lamina of a stipule showing marginal growth and differentiation of the different tissues. Figs. 22 and 23 (each × 500) and Fig. 24 (× 234). *Smi.*, Submarginal initial; *ad.* and *ab.*, adaxial and abaxial epidermis; *ml.*, middle layer; *vs.*, vascular strand; *Pl.*, palisade tissue; *Sp.*, spongy tissue; *S.epi.*, subepidermal layer.

the abaxial and the adaxial epidermis. The middle layer and to some extent the abaxial subepidermal layer take part in the formation of vascular bundles. The adaxial subepidermal layer differentiates into a single layer of palisade cells and the abaxial subepidermal layer and the middle layer where they do not form vascular bundles become the spongy tissue (Figs. 23 and 24). Cells of both the epidermis form stomata. The mature stipule is thin and membraneous and consists of palisade and spongy tissues.



Text-Figs. 25 to 36.—Serial transections of primordium 6 from the free apex showing the courses of the leaf traces through the base. *Br. m.*, *Br.* m_1 and *Br.* m_2, branches of median and secondary median traces; *Br.* l_1 and *Br.* l_2, branches of lateral traces; *Cp b.*, composite bundle of the secondary median trace and the branch of the lateral trace; *Br. Cp b.*, branching of composite bundle; *Pt.*, petiole and *St.*, stipule. Fig. 25 is 1100 μ below the free apex; Fig. 26 is 80 μ above Fig. 25; Fig. 27 is 50 μ above Fig. 26; Fig. 28 is 20 μ above Fig. 27; Fig. 29 is 90 μ above Fig. 28; Fig. 30 is 20 μ above Fig. 29; Fig. 31 is 70 μ above Fig. 30; Fig. 32 is 60 μ above Fig. 31; Fig. 33 is 30 μ above Fig. 32; Fig. 34 is 40 μ above Fig. 33; Fig. 35 is 410 μ above Fig. 34 and Fig. 36 is 190 μ above Fig. 35 (each × 18).

Text-Fig. 37.—Split half of the nodal region of a matured leaf after clearing with chloral hydrate showing the longitudinal courses of the median and one lateral trace through the internode, petiole and the stipule (× 8). *mr. st.*, midrib of the stipule; *Ps. st.* and *An. st.*, posterior and anterior wings of the stipule.

Courses of the leaf trace bundles in the base of the leaf and the vascular system of the stipules

In connection with the origin of the stipules the courses of the three trace bundles in the base of the leaf have been described in a general way. In this section (Figs. 25 to 36 and 37) they are followed in the bases of the adult leaves, in a little more detail. We have seen that the base 'grows vertically for some distance congenitally united with the axis. As a matter of fact it forms a heavy mantle on the axis (Leaf-skin of Saunders ?). In this mantle the three bundles move up, the laterals gradually diverging tangent-wise and the median radius-wise. The upper end of this mantle is not perfectly horizontal but oblique as the separation of the base from the axis starts from its extreme ends after the laterals have changed their courses towards the central region, until higher up in the axis it is completed. In the mantle the median divides into two secondary medians m_1 and m_2; m_2 divides again and the branches move towards the sides (Figs. 13 to 15, 17 to 19, 25, 26 and 37).

Meanwhile, the laterals during their oblique vertical courses through the mantle proceed giving off branches to the separating arms of the base, and these branches cause great development of the anterior wing of the stipule (Figs. 1 *b*, 4 *a* and 37). After giving off branches they continue their oblique courses through the base until they unite with the nearest secondary medians (Figs. 27 and 37). This happens before the base completely separates from the axis. A branch or two from this united strand on each side of the central region, go to the posterior wing of the stipule and form its vascular system. The vascular supply of a stipule is thus derived from two sources. The petiole on the other hand gets its vascular supply from the median and its branches, and from a branch of each of the laterals. This unequal distribution of the branches of the laterals and the median, perhaps, has something to do with the expression of the stipules and the adult leaf in *Pisum*.

Discussion

The present studies on the development of the leaf of *Pisum sativum* support Eichler's view that *Pisum* falls into Trécul's basifugal group but do not support his further contention that the stipules appear as lateral structures of the first order. On the other hand they show that the stipules and the leaflets belong to different categories. We have noticed and others also have reported before, that the primordium soon after its emergence is differentiated into two regions (lower and upper) which behave differently as they grow and develop. The leaflets are products of the 'upper

region' and originate as papillate outgrowths by periclinal divisions which start in the subepidermal cells of the adaxial surface and later extends into underlying cells. The stipules on the other hand have no separate primordia but are brought into existence by the upward growth of the extending lateral arms or wings of the base on the two sides of the base of the petiole. The upward growth of the stipules is caused and maintained by the laterals and their branches, and from the bundles produced by the united branches of the laterals and the secondary medians.

The base is characterised by a very great marginal activity of its extending lateral arms; the cells derived from this marginal activity differentiate into palisade and spongy tissues which characterise the foliar nature of the stipules. The base is for the most part adnate to the axis and is obliquely free from the latter at its upper end only for a very short distance. The laterals which are at greater tangential divergences from the median, near the upper end of the base, turn obliquely towards the petiolar region of the base and give off branches during their oblique vertical courses. These branches and the branches given out by the united courses of the branches of the laterals and the secondary medians form the vascular system of the stipules. The two wings of the stipules (posterior and anterior) receive vascular supplies from two different sources and develop unequally. Due to the very little extent of the free base, the stipules in their adult morphology appear cauline in nature.

The tendency of branching of the laterals in connection with the formation of the stipules is inherent and the phenomenon can very well be compared to the development of the leaf primordium itself at the shoot apex where the former is initiated before its median bundle coming from the axis causes its emergence. Moreover, if there had been no branches from the laterals the base would have maintained its sheathing character like what we see in *Heracleum* (Majumdar, 1942 and 1948). It is clear, therefore, that the behaviours of the laterals during their courses through the base towards the petiole determine the extent and nature of the base: if unbranched, *sheathing*, if branched, *stipular*.

Summary

The foliar primordium is initiated in a particular sector of the apical dome by the anticlinal divisions in the two tunica layers (t_1 and t_2). Simultaneous periclinal divisions in the inner tunica layer (t_2) and the outermost layer of corpus cells in this region result in the papillate outgrowth on the surface of the free apical dome. Just below the emergence of the primordium

prodesmogen strand (procambial initials) differentiates in the corpus derivatives and becomes continuous with the acropetally developing desmogen strand (procambium strand) coming from the axis. The desmogen strand in continuity with the prodesmogen strand becomes the median bundle of the primordium. Two more bundles of the trace from the axial ring (trilacunar node) enter the base a little later and influence further growth and development of the primordium.

Early in the ontogeny, the primordium (the fore limb) differentiates into an 'upper region' with a pair of papillary outgrowths on its adaxial surface (the leaflet primordia) and a 'lower region' (the base) with extending lateral arms. Papillate outgrowths arise acropetally on the 'upper region' by periclinal divisions in the subepidermal layer which later extends into underlying cells. The terminal region and subterminal pair or pairs of papillæ are transformed into tendrils.

The 'lower region', *i.e.*, the base which shows radial extension of its central region and tangential extension of its lateral arms around the axis, remains for the most part adnate to the axis and is free from the latter at its upper end, only for a very short distance. From the axis the base begins to separate from its lateral ends while the two are elongating together and the separation is completed a little higher up the stem. The base now being free resolves into three parts. The central region becomes the petiole and the two extending lateral arms develop directly into the stipules. It is, thus, seen that the stipules have no separate primordia like the leaflets but they are direct upward divergences of the lateral arms of the base. Owing to the very little extent of the free base and to the oblique insertion of the stipules they appear cauline in nature.

The vascular supplies to the two wings (posterior and anterior) of a stipule are derived from two sources. The anterior wing gets all its supply from the laterals earlier, while the posterior wing gets one or two branches from the united bundle of a branch of the lateral and a secondary median later, with the result that the two wings develop unequally. The courses of the leaf trace bundles through the base have been described in detail.

The marginal activity of the stipules is very great and the cell lineages derived from the submarginal initial differentiate ultimately into vascular bundles and the palisade and the spongy tissues.

Not only in size, but also in tissue differentiation, vascular supply and mode of origin the stipules of *Pisum sativum* differ from the types hitherto investigated and reported.

The behaviour of the laterals in their passage towards the petiole determine the nature and extent of the base. If they remain unbranched the base is *simple* or *sheathing*, if they branch, the base is *stipular*.

The work has been done under the guidance of Prof. G. P. Majumdar, PH.D., F.N.I., Botany Department, Presidency College, Calcutta.

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THE EMBRYOLOGY OF *JUNCUS PRISMATOCARPUS* BR. AND *J. EFFUSUS* LINN.[1]

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Introduction

A search through the available literature indicates that comparatively little work has been done on the embryology of the family Juncaceæ. The most important contribution is that of Brenner (1922) who studied a few species of *Juncus* and *Luzula* and one species of *Distichia* and *Oxychlæ*. More recently, Souèges (1923) has studied the embryonal development in *Luzula* and *Juncus*, and Wulff (1939) has given an account of the development of the pollen grain in *Juncus filiformis*, *J. bufonius* and *J. squarrosus*. The relationships of the family still remain undecided. Comparisons have been made at different times with the Helobiales, Cyperaceæ and Liliaceæ but without any definite conclusions.

Material and Methods

A few years ago, Mr. H. Gangulee, then a research student in this University, collected from Shillong some material of *J. prismatocarpus* but for unavoidable reasons he was unable to proceed with the work. At the request of Prof. P. Maheshwari, Mr. R. N. De very kindly sent some further material of this species as well as of *J. effusus*. The flowers and fruits of both species had been microtomed in the usual way by Prof. Maheshwari and passed on to me for investigation soon after I joined the Dacca University in 1948.

The slides were stained in iron-hœmatoxylin and safranin-fast green. Of these, the former proved more satisfactory for most purposes, but the latter combination was also useful in bringing out the cell walls and served as a check in the interpretation of some stages.

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[1] A preliminary report of this paper appeared in the *Proceedings of the Indian Science Congress* (Zaman, 1949).

The Flower

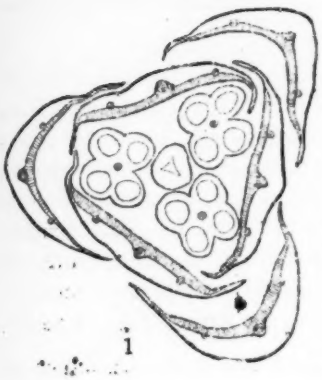
The flowers are slightly protandrous and arise in small greenish clusters. The inconspicuous perianth comprises six members arranged in two whorls, both greenish and suggestive of wind pollination. Each of the three anthers is two-celled (Fig. 1), basifixed and introrse, and opens lengthwise. The gynœcium is tricarpellary. The ovary is unilocular, with three parietal placentæ each bearing an indefinite number of ovules arranged in two rows (Fig. 3). The fruit is a loculicidally dehiscing capsule. The perianth lobes are stiff and coriaceous. The stomata lie on the outer epidermis, below which there are a few layers of loose chlorenchymatous cells followed by a thick-walled sclerenchymatous tissue (Fig. 2). There are three vascular bundles both in the outer and in the inner perianth lobes.

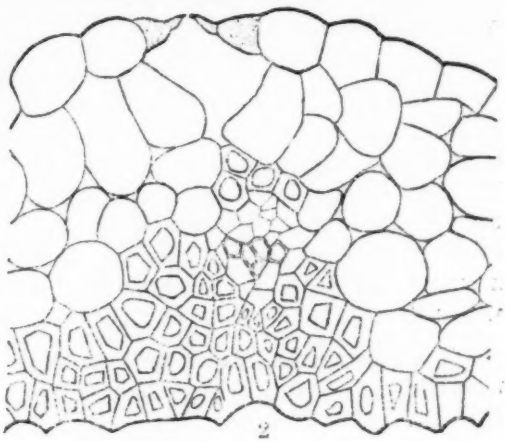
Microsporogenesis and Male Gametophyte

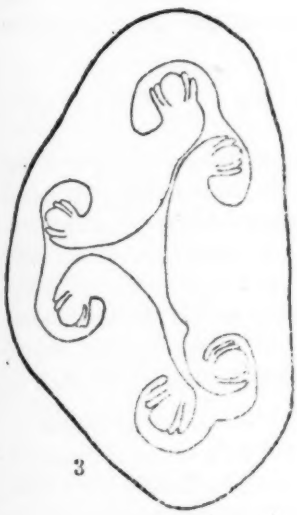
The earliest stages at my disposal showed the anther wall differentiated into the epidermis, endothecium, one middle layer and the tapetum (Fig. 5). The former becomes thin and inconspicuous at maturity while the endothecium develops the usual fibrous thickenings and forms the main parietal layer in the anther (Fig. 12). In some anthers and at certain points the endothecium was found to be two-layered.[2] In some of the sections I was unable to observe any fibrous thickenings which suggests the probability that under certain conditions the flowers may be cleistogamous. According to Brenner (1922) the middle layer persists and can be seen even after the dissolution of the tapetum, but I could not find any evidence of this in my material, nor can I support his statement that the tapetum is cut off from the sporogenous cells. My observations in this respect agree with those of Wulff (1939) in that the middle layer is ephemeral and the tapetum has a parietal origin like the other wall layers.

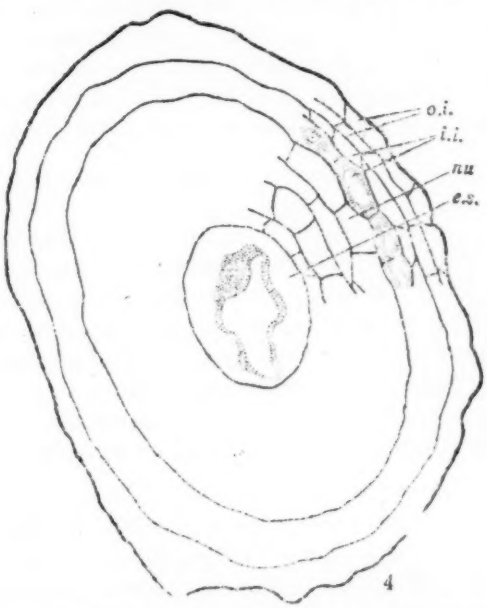
Brenner (1922) noticed occasional periclinal divisions in the tapetum so that it becomes two-layered at places, and also mentions having seen a few binucleate cells. In my material the tapetum was composed of a single layer of uninucleate cells having one prominent nucleolus (rarely two) and staining only a little less densely than the microspore mother cells. According to Wulff the degeneration of the tapetum sets in at the time of formation of the generative cell and it is completely dissolved by the time the division of the generative cell begins. In my material it was found to persist a little

[2] Brenner also draws a figure of *Oxychlœ andina*, showing what he regards as a fragment of the middle layer which has developed thickenings similar to those seen in the endothecium. This may perhaps explain the occurrence of a two-layered endothecium in some of my sections.









FIGS. 1–4. Fig. 1. T. S. flower bud showing perianth, anther lobes and triradiate stylar canal. The lower sclerenchymatous portion of the perianth lobes has been represented by short parallel lines, × 54. Fig. 2. T. S. part of a perianth lobe showing stomata, vascular bundles, and the sclerenchymatous tissue, × 1000. Fig. 3. T. S. ovary, × 175. Fig. 4. T. S. ovule showing the outer and inner integuments (*o. i.* and *i. i.*) nucellar tissue (*nu*) and embryo sac (*e. s.*) × 533.

longer and, before dissolution, prominent vacuoles were seen near the lateral walls of the cells.

I was unable to trace the development of the pollen in such detail as Wulff has done, but several stages seen by me corroborate his account in all essential respects. As in the species studied by him, I also found the microspore mother cells to be comparatively larger than the megaspore mother cells (*cf.* Figs. 6 and 13). A maximum of six to seven microspore mother cells are seen in a transverse section of an anther lobe (Fig. 5). The divisions of the microspore mother cells are simultaneous and the microspores are arranged tetrahedrally (Fig. 7). Their subsequent development continues without a separation of the four cells composing the tetrad (Figs. 8 to 11).

The generative cell is cut off towards the inner angle of each cell of the tetrad (Fig. 8).[3] But it soon changes its place so that the two male cells formed from its divsions (Figs. 9, 10) do not always occupy the position in which the generative cell was laid down. The cytoplasm around the male nuclei is quite clear in these preparations and is especially thick at the two ends so that the male gametes have a lens-shaped appearance (Fig. 11). In the mature pollen grain the cytoplasm stains so densely that the nuclei can be seen only in very thin and well stained sections.

Ovary and Ovules

As mentioned before, the ovary is tricarpellary with very well-developed inwardly protruding placentæ (Fig. 3). There is considerable variation in the family with regard to this feature, some species having well-developed parietal placentæ and a unilocular ovary (*J. lamprocarpus*), and others with massive placentæ meeting at the centre so as to give rise to the trilocular condition (*J. compressus* and *J. glaucus*). *J. bufonius* and *J. leersii* show an intermediate condition, the placentæ being fused below but free above. These features have been noted by Buchenau (quoted in Brenner, 1922).

The ovary wall is composed of four layers of cells of which the outermost is heavily cutinised on the outer surface. The style is only about half as long as the ovary. A cross-section through it shows triradiate lacuna lined with richly protoplasmic cells. The stigmas are long and beset with thickly studded papillæ.

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[3] Gœbel (1933) thought that the generative cell is always cut off towards the outer side of the pollen grain but Geitler (1935) showed that the reverse is more frequent. He also claims that the position of the spindle giving rise to the tube and generative cells has a systematic significance. For further details see Wulff and Maheshwari (1938) and Maheshwari (1949).

The ovules are anatropus and bitegmic, at first growing transversely to the long axis of the ovary and then bending downwards. Brenner says that the bending of the ovules takes place irregularly depending on spatial factors, and the cells, which line the micropyle, are considerably swollen as compared to the rest. In my material this irregularity in the bending of the ovules was observed only rarely. Each of the integuments is two-layered. At the eight-nucleate stage of the embryo-sac, the inner layer of the inner integument begins to get thickened and shows a deposit of some red-staining material whose exact nature I have been unable to determine (Fig. 4).

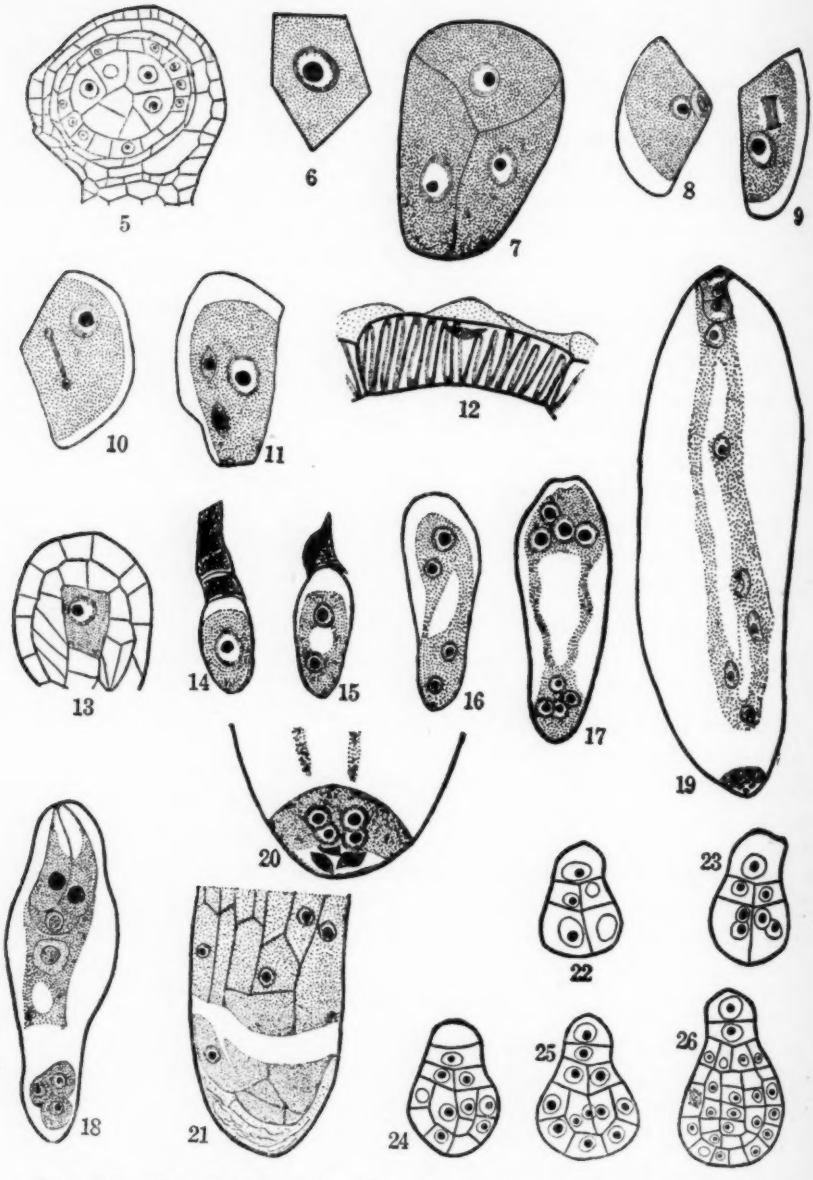
Megasporogenesis and Female Gametophyte

The young nucellus often gives the impression of having two or three hypodermal archesporial cells. Only the one occupying the middle position divides into the primary wall cell and the megaspore mother cell. The latter is often mounted on an axial row of two or three cells (Fig. 13) which stain densely and look like sporogenous cells. According to Brenner "in der Gattung *Juncus* kommt keine weitere Zellschicht zwischen der Epidermis und der Embryosackmutterzelle resp. dem Embryosack zustande," although *Luzula* shows a periclinal division of primary wall cell.

Megaspore formation takes place in the normal way and of the four megaspores composing a linear tetrad it is the lowest which functions and gives rise to the embryo sac (Fig. 14). The three uppermost megaspores begin to degenerate before the division of the functioning megaspore nucleus (Figs. 15-18) and it has the usual organisation characteristic of the Polygonum type (Maheshwari, 1948) (Fig. 18). As in *J. bufonius* (Brenner, 1922), the synergids are fairly large. The polar nuclei fuse in the middle of the embryo sac to form a large secondary nucleus. The three antipodal cells are much smaller than the cells of the egg apparatus but can be easily recognised during the earlier stages of the development of the embryo (Figs. 19, 20). Some of the embryo sacs degenerate and their nuclei become crowded together in the centre.

Fertilization

Brenner (1922) mentions the difficulty in studying the stages associated with fertilization due to the poor stainability of the nuclei discharged by the pollen tube. I was also unable to make any direct observations on fertilization in the material that was at my disposal. However, the presence of a pollen tube and two nucleoli in the egg cell in some embryo sacs, and of only one nucleolus in the egg cell of ovules without pollen tubes makes it seem probable that fertilization takes place in the usual way.



FIGS. 5–26. Fig. 5. T. S. anther lobe showing epidermis, endothecium, middle layer, tapetum and microspore mother cells, × 420. Fig. 6. A microspore mother ceil × 1000. Fig. 7. Tetrad of microspores, × 1000. Fig. 8. A single pollen grain from the tetrad

showing cutting off of generative cell in the inner angle of the microspore × 1000. Fig. 9. Pollen grain showing generative cell in anaphase, × 1000. Fig. 10. Pollen grain showing completion of division of generative cell, × 1000. Fig. 11. Mature pollen grain showing the vegetative nucleus and the lens-shaped male cells, × 1000. Fig. 12. T. S. part of anther lobe showing fibrous endothecium. The remains of the epidermis are also seen, × 630. Fig. 13. L. S. ovule showing megaspore mother cell, × 420. Fig. 14. Linear tetrad of megaspore, × 1000. Fig. 15. Two-nucleate embryo sac, × 1000. Fig. 16. Four-nucleate embryo sac, × 1000. Fig. 17. Eight-nucleate embryo sac × 1000. Fig. 18. Mature embryo sac. The polar nuclei have fused to form the secondary nucleus, × 1000. Fig. 19. L. S. embryo sac showing two-celled embryo and Helobial endosperm. The micropylar chamber contains several free nuclei while the chalazal chamber is binucleate. The antipodal cells are degenerating, × 358. Fig. 20. The chalazal end of an embryo sac showing four free nuclei in the chalazal chamber; note the degenerating antipodal cells, × 1000. Fig. 21. Chalazal chamber in process of degeneration, × 1000. Figs. 22–26. Stages in development of the embryo, × 533.

Endosperm

After fertilization the embryo sac enlarges considerably. Concurrently with this there are also some changes in the nucellar tissue. During the maturation of the embryo sac the nucellus consists of three to four layers of cells at the sides of the embryo sac, two layers at the micropylar end and a more massive tissue at the chalazal end. In later stages the inner of the laterally situated layers degenerates while the cells of the outer become enlarged and vacuolate.

The primary endosperm nucleus moves to the chalazal end of the sac and its first division results in the formaton of two chambers of which the micropylar is much larger than the chalazal (Figs. 19, 20).[4] The latter is very conspicuous because of its dense cytoplasm. Indeed, as Brenner says, if one is not aware of the orientation of the ovule, it may easily be mistaken for the egg which lies at the opposite end and is often less conspicuous. Several free nuclear divisions take place in the micropylar chamber which shows a peripheral layer of lightly stained cytoplasm with the nuclei embedded in it. Later, wall formation takes place and the cells become densely filled with starch grains. At the two-celled stage of the embryo four free nuclei are seen in the chalazal chamber with the antipodals still recognisable at its base (Fig. 19). The four nuclei divide once again producing eight nuclei which become separated by walls. By the aggression of the endosperm cells of the micropylar chamber they are, however, gradually crushed and disorganised (Fig. 21). Eventually the basal cells of the micropylar chamber are also crushed owing to pressure from the upper cells. At this stage the micropylar

[4] Brenner (1922) notes that in *Luzula* no distinct wall is laid down between the basal and the micropylar endosperm chamber.

part of the endosperm also stains more densely, somewhat like the cells of the chalazal chamber in their earlier stage.

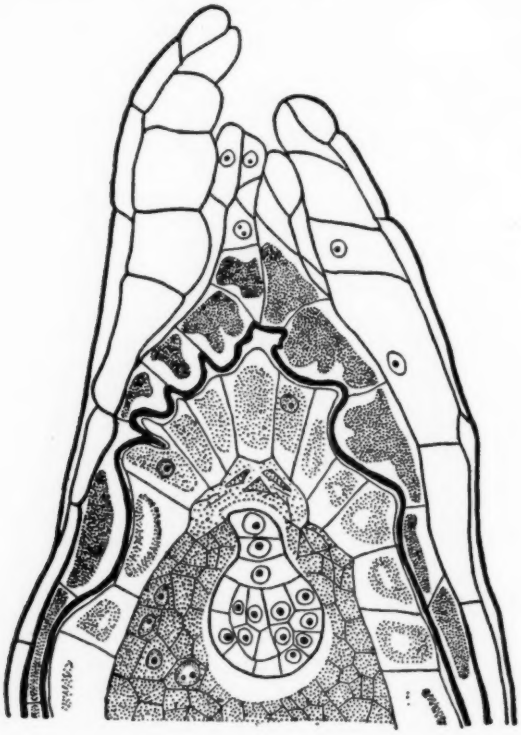


FIG. 27. L. S. micropylar part of mature seed showing the glandular nature of the inner layer of outer integument, and the thickened inner walls of cells belonging to the inner layer of inner integument × 533.

EMBRYO

The fertilized egg divides transversely into two cells (Fig. 19) of which the basal divides again in the same plane resulting in a row of three cells. The terminal cell divides by a wall parallel to the long axis of the embryo sac to form two juxtaposed cells (Fig. 22) which undergo another vertical division to give rise to quadrants (Fig. 23). The second cell also divides transversely to form the two cells of the suspensor (Figs. 24-26). Older embryos show the root tip, lateral stem tip and prominent cotyledonary sheath. The embryo seems to digest the surrounding cells of the endosperm with its secretions and is later seen in an empty space without any apparent connection with the endosperm (Fig. 27). The cells of the endosperm lack

functional nuclei at this stage and are all gorged with starch grains excepting those which are in the immediate vicinity of the growing embryo.

Seed

During the maturation of the seed, the ovule shows a number of changes. The outer layer of the outer integument is gradually absorbed and is practically absent in the seed. The inner layer of the outer integument becomes glandular at the micropylar end. The outer layer of the inner integument is also absorbed except at the top. The cells of the inner layer of the inner integument show a conspicuous thickening of their inner (*i.e.*, towards the nucellus) walls and the contents take up a red colour. It may be noted that I did not come across any "mucilaginous tissue blocking the micropyle" as described by Laurent (1903) in *J. bufonius*. My sections show clearly that the micropyle is lined by the cells of the inner layer of the inner integument. The apical cells of the nucellar epidermis elongate radially, the walls remaining thin (Fig. 27). A hypostase consisting of thick-walled cells with brownish contents develops at the base of the nucellus.

Discussion

The systematic position of the Juncaceæ is still controversial. In the first place it shows some important resemblances with the Liliaceæ. The flower is built on exactly the same plan as in the latter, the floral formula being P_3+_3, A_3+_3, or $G_{(3)}$. Certain genera with a membranous perianth like *Xanthorrhœa* and *Calectasia*, are included indifferently in either family. The only important differences are that in the Liliaceæ the perianth is usually well developed and brightly coloured. In the Juncaceæ, on the other hand, it is inconspicuous and coriaceous and the flowers are anemophilous. In the Liliaceæ the endosperm is usually without starch but in the Juncaceæ starch is always present.

Resemblances with the Glumiflorae are seen chiefly in the general habit, in the nature of coriaceous and inconspicuous perianth and in the presence of starch in the endosperm.

On the basis of these characters some botanists (Wettstein, Engler and Rendle) consider that by gradual elaboration the Juncaceæ have given rise to the Liliaceæ, the greatest modifications being exhibited by the perianth.

Others (Arber and Hutchinson) hold that the Juncaceæ have been derived from a Liliaceous stock by a gradual reduction and that they show a distinct tendency towards the evolution of more reduced forms like the Restionaceæ and Glumifloræ.

So far as the embryological evidence is concerned, the Juncaceæ show marked resemblances in the development of the ovule, embryo sac, endosperm and embryo with several members of the Liliaceae. The chief difference is in the development of the pollen grains which mature separately in the Liliaceæ but are held together in tetrads in the Juncaceæ. Wulff lays much importance on this point and compares the Juncaceæ with the Cyperaceæ, but while all the four microspores are functional in the former, only one matures in the latter. Regarding the development of the embryo sac, endosperm and embryo, our knowledge of the Cyperaceæ is extremely meagre and further work is necessary before any opinion can be given on the relationships of the Juncaceæ and the Cyperaceæ.

Summary

1. The inner surface of the perianth lobes is heavily lignified and stomata are borne on the outer surface only. The flower is hermaphrodite. The ovary is unilocular and bears three parietal placentæ. The ovules, which are anatropous, are arranged in two rows on each placenta.

2. The anthers are four-lobed. The microspore mother cells are surrounded by the uninucleate cells of the tapetum, the middle layer, endothecium and epidermis. The pollen grains are united in tetrads. The generative cell is cut off towards the inner side of each cell of the tetrad and ultimately divides to form two male cells. The endothecium usually shows fibrous thickenings.

3. The ovules are bitegmic. A hypodermal cell of the nucellus becomes differentiated as the primary archesporial cell which divides to form a parietal cell and the megaspore mother cell. This undergoes the usual meiotic divisions producing four magaspores of which the chalazal functions and the other three soon degenerate.

4. The development of the embryo sac is of the Polygonum type.

5. The endosperm is of the Helobial type. The nucleus of the chalazal chamber divides twice to form four free nuclei which divide once more to produce eight nuclei. In the micropylar chamber several free nuclear divisions take place followed by wall formation. The chalazal portion of the endosperm is crushed by the growing micropylar portion.

6. The embryogeny is similar to that of *Luzula* previously studied by Souèges.

7. In the mature seed the endosperm cells lack functional nuclei and are gorged with starch grains.

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It gives me great pleasure to record my deep sense of gratitude to Prof. P. Maheshwari from whom I received guidance and encouragement during the preparation of this work. I also feel indebted to him for his magnanimity in giving over to me the material, microscopic preparations and notes of his own observations upon which this paper is based. I also owe a debt to Dr. B. M. Johri for revising the proofs of this paper.

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STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part V. Placentation in the Vespertilionid Bat—*Scotophilus wroughtoni* (Thomas)

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(With Seven Plates)

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1. Introduction

The early development and implantation in *Scotophilus wroughtoni* has been described in an earlier paper (1949 *b*). It is my purpose in this paper to describe the development of the fœtal membranes and the structure of the placenta in this species.

The embryology of Microchiroptera has received very little attention from the embryologists. Out of the 16 families of microchiroptera, species belonging to only a few families have been studied and even in these cases details regarding certain aspects of placentation are lacking.

Van Beneden (1888) working on the development of *Vespertilio murinus* (Vespertilionidæ) found that the endothelium of the maternal vessels is lost in the placenta. Thus he recognised that the placenta is of the hæmochorial type. Duval (1896) redescribed the placenta of *Vespertilio murinus* and restated the fact of the loss of the endothelium of the maternal capillaries resulting in a direct contact of the maternal blood and the trophoblast. While van Beneden (1888) attributed a maternal origin to the trophoblast, Duval (1896) made it clear that the trophoblast is fœtal in origin. Branca (1927) described the histological structure of the placenta of *Vespertilio murinus* and mentioned that the maternal endothelium is not completely lost but persists as an enucleate cytoplasmic membrane, so that the final structure of the placenta is not strictly hæmochorial. Gérard (1928 *a*) stated that in *Vesperugo noctula* (Vespertilionidæ) the yolk-sac attains communication with the uterine lumen on the mesometrial side by the formation of an umbilico-uterine orifice. In another paper (1928 *b*) he mentioned that in *Vesperugo noctula* also the endothelium of the maternal capillaries persists as a cytoplasmic membrane as in the case of *Vespertilio murinus* described by Branca (1927). Kempermann (1929) working on *Miniopterus schreibersii* (Vespertilionidæ), found the complete loss of the maternal endothelium in the placenta and also found the trophoblast directly bathed in the maternal blood—an observation quite different from that of Branca (1927) and Gérard (1928 *b*). Van der Sprenkel (1932) in his monographic work on the persistence of the yolk-sac in the embryos of bats, stated that the yolk-sac persists in some form or other till term and also stated that it functions as a gland of internal secretion during later stages of gestation. He found the existence of a real, functional "Omphalo-placenta" in *Vesperugo noctula*, *Rhinolophus hipposideros* (Rhinolophidæ) and *Hipposideros* (Hipposideridæ). Thus he observed regarding *Hipposoderos* CI—'Der Döttersack hat eine sehr ausgiebige Dötterplacenta entwickelt, welche fast die ganze Inneroberflasche des Uterus einnimmt, das Omphalo-chorion tragt viele Zolten, welche stark vascularisiert sind

und in die Krypten des endometriums eintanchen' (page 214). Ramaswami (1933) described the chorioallantoic placenta of *Vesperugo leisleri* (Vespertilionidæ) to be of a hæmochorialis type and also confirmed the persistence of the yolk-sac till term, though, he mentioned, that in certain parts the yolk-sac cavity occurs as a small space, and it is absent in certain other parts during later stages of development. In a small paper, Hamlett (1934) mentioned that in the case of *Molossus crassicaudatus* (Molossidæ) there is a bicornuate uterus with pregnancy only in the right horn. The placenta in this species is endotheliochorial in nature. He also mentioned that the yolk-sac cavity though large does not come into direct contact with the chorion but lies free in the extra-embryonic cœlom. His observations on *Molossus crassicaudatus* were confined to a single specimen. In a later paper (1935), he confirmed his earlier (1934) observation on *Glossophaga soricina* (Phyllostomidæ) that there is a simplex uterus with fundal implantation, and that the placenta is hæmochorial. He recorded also the curious fact of the formation of a decidua capsularis in *Glossophaga soricina*. He mentioned the early disappearance of the yolk-stalk at as early a stage as the 2·5 mm. embryo. The bilaminar omphalopleure persists till term, and the yolk-sac cavity is reduced to a minimum. Mossman (1937) found that in *Nyctinomus cynocephala* (Molossidæ) there is an early disappearance of the bilaminar omphalopleure and the formation of a well developed chorio-vitelline placenta which continues to bear villi up till the limb-bud stage. He described the chorioallantoic placenta as 'endotheliochorial or hæmochorial'. Wislocki and Fawcett (1941) described the placenta in *Artibeus jamaicensis parvipes* (Phillostomidæ) and stated that as in the case of *Glossophaga soricina* (Hamlett 1934 & 1935), the uterus is simplex, the implantation fundal, the yolk-sac large and persistent, and the chorio-allantoic placenta hæmochorial. Wimsatt (1945) in his excellent work on the placentation on *Myotis lucifugus lucifugus* (Vespertilionidæ) described a large yolk-sac which attains great functional importance during the early stages of development. Later the chorio-vitelline placenta is destroyed by the extension of the exocœlom and the consequent invagination of the roof of the yolk-sac. Wimsatt calls this condition as an example of incomplete or partial inversion of the yolk-sac. He questions the correctness of the phrase 'inverted yolk-sac placenta', since the vascular invaginated segment of the yolk-sac never comes into direct contact with the maternal tissue because of the persistence of the bilaminar omphalopleure.

2. Material and Methods

Details of collections have been given in Parts I and II and III of these studies (Gopalakrishna, 1947, 1948 and 1949 *a*). Pregnant uteri were fixed

in Bouin's fixative. In cases of advanced conceptuses the uterine wall was punctured or slightly slit open with a view to allowing the penetration of the fixative. In cases of early stages of pregnancies the gravid uteri were cut at 10 μ, but in the cases of advanced pregnancy only the placenta was cut at 10 μ. Sections were stained in Ehrlich's hæmatoxylin and counter-stained with Eosin.

3. Observations

A. *The Implanted Blastocyst* (Plate XIII, Fig. 1)

After implantation the blastocyst can be distinguished into an embryonic and an extra-embryonic region. The former consists of an embryonic disc made up of three or four layers of cells underlined by the entoderm consisting of a single layer of fusiform cells. The embryonic disc is arched over by the amnion, which is in contact with the basal cytotrophoblast. The embryonic disc, therefore, forms the floor of the amniotic cavity.

The extraembryonic part of the blastocyst consists of the large yolk-sac which fills most of the uterine lumen and extends up to the mesometrial side of the uterus. A small part of the uterine lumen on the mesometrial side persists as small cavity, into which the uterine glands open by wide mouths. The yolk-sac cavity is lined internally by the vitelline entoderm.

On the antimesometrial and lateral sides the trophoblast has penetrated into the uterine endometrium engulfing the maternal capillaries which have lost their endothelium in certain parts (Plate XIII, Fig. 2). The penetrating trophoblast has become multi-layered and syncytial while the basal cyto-trophoblast alone shows the cell boundaries. The distal wall of the yolk-sac, *i.e.*, the yolk-sac wall towards the mesometrial side, remains bilaminar.

The further development of the embryo consists of a series of events occurring in the following sequence—the differentiation of the mesoderm, the establishment of the vascular chorio-vitelline placenta, the later obliteration of this by the extension of the exocœlom and the expansion of the amniotic cavity, the development of the allantois, and the final structural modifications of the chorio-allantoic placenta. While these events occur in the above-mentioned sequence, there may be a telescoping of events so that at any one stage more than one event may be taking place. I shall preceed to describe these stages in the strictest order of the sequence of events.

B. *Stages leading to the formation of the Neural Groove*

(*a*) *Stage I*—(Plate XIII, Fig. 3).—The embryonic disc shows the formation of the neural groove and the mesoderm. The latter lies separated from the ectoderm by a clear space. The mesodermal plate is made up of a mass

of cells loosely arranged. The entoderm occurs as a thin layer of fusiform cells. The mesoderm extends laterally towards the trophoblast which is closely applied to the uterine endometrium, and invades it. The maternal vessels are surrounded by this invading trophoblast and in most parts the endothelium of the maternal vessels is lost (Plate XIV, Fig. 4).

The embryonic disc is arched over by the amnion which is closely applied to the cytotrophoblastic layer on the antimesometrial side. Only on the sides of the amniotic cavity has the amnion been reinforced by the mesoderm.

The yolk-sac cavity is large and occupies the whole of the uterine lumen except on the mesometrial side, where the persistent bilaminar omphalopleure is not in contact with the uterine tissue (Plate XIII, Fig. 3). The yolk-sac wall can be distinguished into three histologically distinct regions—the embryonic part (*i.e.*, the roof of the yolk-sac), the lateral walls and the bilaminar omphalopleure. On the embryonic part the yolk-sac wall is made up of a layer of entodermal cells supported by two or three layers of mesodermal cells (Plate XIV, Fig. 4). As we pass towards the lateral sides, the yolk-sac wall becomes three layered because of the extension of the mesoderm between the entoderm and the trophoblast (Plate XIII, Fig. 3). The mesoderm extends only for a short distance. The lateral walls of the yolk-sac forms true placental relationship with the uterine endometrium because the trophoblast has invaded the endometrium and has surrounded the lacunæ containing maternal blood. The yolk-sac entoderm is a thin layer of fusiform cells underlying the cytotrophoblast and forms the inner layer of the bilaminar omphalopleure (Plate XIV, Fig. 5).

The basal cytotrophoblast on the antimesometrial and lateral sides of the uterus migrates into the uterine endometrium. The cellular boundary of the penetrating trophoblast cannot be made out as it has become syncytial while cell boundaries are quite clear in the basal cytotrophoblast (Plate XIV, Fig. 4). The trophoblast shows the presence of the villi in some places, so that at different regions, the placental relationship is different, ranging from the syndesmochorial to the hæmochorial complexity. The lateral walls of the uterus is also included in placental formation.

I have referred, in my earlier paper (1949 *b*), to the endometrial changes, in the uterus at the time of implantation. These changes are more pronounced and result in the formation of a loose periplacental zone, already indicating the region of separation during parturition.

(*b*) *Stage II*—(Plate XIV, Fig. 6).—In this stage the neural groove has deepened still further and the mesoderm, which was in the form of a sheet of cells underlying the embryonic ectoderm, shows the beginnings of the

formation of the cœlom. The mesoderm has extended laterally between the yolk-sac entoderm and the trophoblast up to about half of the lateral wall of the yolk-sac, so that in this part the omphalopleure is trilaminar. Angiogenesis has commenced and blood corpuscles are seen in the mesoderm. The upper part of the lateral wall of the yolk-sac is vascularised (Plate XV, Fig. 7). The mesoderm has also extended round the amnion between it and the trophoblast, thus forming an additional layer to the amnion, which is separated from the chorion. The amniotic cavity has increased in extent. The trophoblast has further invaded the maternal endometrium forming large lacunæ containing maternal blood.

The characteristic feature of this stage is the occurrence of the vascular chorio-vitelline placenta on the embryonic hemisphere of the yolk-sac together with the non-vascular yolk-sac placenta towards the lower half of the lateral wall of the yolk-sac.

The abembryonal part of the omphalopleure is bilaminar, and never establishes any placental relationship with the uterus till term.

(*c*) *Stage III*—(Plate XV, Fig. 8).—In this stage the mesoderm has extended further along the lateral wall of the yolk-sac and has reached the margin of the placental cup. The vitelline vessels have also extended further and have vascularised the major part of the lateral sides of the yolk-sac.

The changes observed in the trophoblastic syncytium in the previous stage are augmented. The junctional zone is clearly demarcated, because the uterine endometrium here has become very loose, and contains large number of spaces (Plate XVI, Fig. 9).

The uterine wall as a whole is thick on the anti-mesometrial side and gradually reduces in thickness as we pass towards the mesometrial side, finally becoming very thin on the mesometrial side. On the antimesometrial side some maternal vessels may be seen entering the placental zone.

C. Stage of Allantoic Diverticulum—(Plate XVI, Fig. 10)

The neural groove is closed and the amniotic cavity has become extensive. The allantoic rudiment occurs as a mass of cells arising from the hinder end of the embryo. The allantoic rudiment has not reached the base of the placenta.

At this stage the chorio-vitelline placenta is of great functional importance as evidenced from its structure. The whole of the lateral wall of the yolk-sac is richly supplied with fœtal vessels. The roof of the yolk-sac is drawn away from the uterus as a result of the extension of the exocœlom between the somatic and the splnachnic layers of mesoderm. Only in this region

the yolk-sac placenta does not occur, because the vascular yolk-sac wall is not in contact with the trophoblast.

D. *Chorio-allantoic Placentation Stages*

(*a*) *Stage I*—(Plate XVII, Fig. 11).—The gap between the previous stage and this one is a little wide, since this stage is, in several respects, advanced over the previous stage in development because of—(i) the occurrence of a true chorio-allantoic placenta by the growth of the allantois with its blood vessels across the exocœlum towards the base of the placenta on the anti-mesometrial side, (ii) the presence of the yolk-sac placenta towards only the lower one-third of the lateral wall of the yolk-sac, the upper two-thirds being destroyed by the extension of the exocœlom; thus the chorio-vitelline placenta, and the chorio-allantoic placenta, are both functional, (iii) the reduction of the yolk-sac cavity.

The allantois is disposed towards the anti-mesometrial side and consists of a vascular stalk enlarged at the base of the placenta into a vesicle. The cavity of the vesicle is in continuity with the gut of the embryo. The lumen of the allantoic stalk is lined by columnar-cells, while the extra-fœtal part of the allantoic lumen, including the cavity of the vesicle, is lined by fusiform cells of the allantoic mesenchyme. Thus the vesicle does not have an epithelial lining in the strictest sense of the term. The allantois carries the fœtal vessels to the base of the placenta only on the antimesometrial side, the allantoic mesenchyme and the blood vessels having not yet extended to the lateral sides.

There are also some striking histological changes in the placental trophoblast. At several places the basal cytotrophoblast shows cords of cells penetrating into the syncytiotrophoblast (Plate XVII, Fig. 12), and hence in many places the cytotrophoblastic cords appear to surround the lacunæ formed by the syncytiotrophoblast. As these cytotrophoblastic cords penetrate the syncytiotrophoblast they form large excavations in the syncytium and the allantoic mesenchyme and the fœtal vessels enter into these excavations. The trophoblastic cords at this stage have extended up to about half the thickness of the placenta.

The periplacentary layer is very clear so that the junctional zone between the syncytiotrophoblast and the decidua can be very sharply made out (Plate XVII, Fig. 12). The decidual cells are large and vacuolated, so that the tissue, as a whole, appears loose and spongy under the low power of the microscope.

As a result of the enlargement of the amniotic cavity, and the extension of the exocœlom, and also due to the increased functional importance

attained by the chorio-allantoic placenta, the chorio-vitelline placenta has partly lost its functional importance. At this stage, however, the chorio-vitelline placenta still occurs on the lower part of the lateral wall of the yolk-sac, and the placental surface is profusely supplied with vitelline vessels (Plate XVII, Fig. 12). There is one fundamental difference between the chorio-vitelline placenta and the chorio-allantoic placenta. While in the allantoic placenta the fœtal mesenchyme and blood vessels actually enter the placental complex, the vitelline mesenchyme and vessels never enter the placenta. This may be partly due to the fact that in the region of vitelline placenta the cytotrophoblastic chords are not found to erode into the syncytiotrophoblast. Thus the chorio-vitelline placenta never attains the structural complexity attained by the chorio-allantoic placenta.

The yolk-sac maintains its size, but on the fœtal side it narrows considerably so that its communication with the fœtus is almost stalk-like.

Thus at this stage both chorio-allantoic placenta and chorio-vitelline placenta are functional.

The condition of the uterus with reference to the chorio-vitelline and the chorio-allantoic placenta has already been described. The uterine wall has become thin on the mesometrial side, and the uterine glands, which are very few, have very narrow lumina.

(*b*) *Stage II.*—The exocœlom extends laterally still further resulting in the further obliteration of the chorio-vitelline placenta. There is a distinct change in the shape of the yolk-sac. It has lost its circular outline, and has become very much smaller because the roof of the yolk-sac is pushed towards the mesometrial side by the enlargement of the amniotic cavity.

The allantoic mesenchyme has extended slightly towards the lateral sides, and the allantoic vesicle has also enlarged, and it extends in a fan-shaped manner below the placenta. The allantoic vesicle maintains its connection with the fœtal gut as evidenced by an examination of the serial sections.

There are, however, marked changes brought about in the histology of the placenta. The cytotrophoblastic cords have penetrated still further into the syncytiotrophoblast, forming large excavations at the basal region of the placenta. The cavities thus formed contain the allantoic mesenchyme and fœtal vessels (Plate XVII, Fig. 13). The placenta appears to be made up of columns of cytotrophoblastic cells encircling lacunæ containing meternal blood. In some parts the original syncytium is reduced to a thin membrane intervening between the maternal blood and the cytotrophoblast.

(*c*) *Stage III*—(Plate XVII, Fig. 14).—In this stage the amniotic cavity has enlarged in extent and also the exocœlom. The yolk-sac splanchnopleure

is completely separated from the placenta so that the chorio-vitelline placenta ceases to exist anywhere. The cavity of the yolk-sac has been reduced and occurs as a slit due to the invagination of the vascular roof of the yolk-sac over the persistent bilaminar omphalopleure.

The whole of the placenta is vascularised by the allantoic vessels alone, the allantoic mesenchyme having extended up to the lip of the placental cup carrying with it the fœtal vessels. The cavity of the allantoic vesicle is reduced and the allantoic stalk has become solid and carries fœtal blood vessels. The allantoic vesicle is therefore no more in continuity with the fœtal gut.

The histological changes in the placenta consist in the further augmentation of the changes seen in the previous stage. The cytotrophoblastic villi have eroded further into the syncytiotrophoblast, creating, as they penetrate, large excavations into which the allantoic mesenchyme and fœtal blood vessels enter (Plate XVIII, Fig. 15). The shape of the placenta is distinctly cup-shaped, only the mesometrial part being free from placental formation.

The yolk-sac has lost all its placental significance and has become completely replaced by the allantoic placenta.

(*d*) *Limb-bud Stage.*—This stage is characterised by the appearance of limb-buds on the fœtus. I have in my collection two stages of limb-bud stage; in one there is the formation of only the fore limbs, and in the other both the limbs are formed. The histological details of the placenta in the two stages vary markedly—the latter showing more complex structure than the former. For the sake of convenience I shall describe these two stages together.

There is a progressive reduction in the yolk-sac cavity, so that in the later limb-bud stage it is reduced to a mere slit. But the distinction of the vascular splanchnopleure and the bilaminar omphalopleure is quite clear (Plate XVIII, Fig. 16). The yolk-stalk becomes a solid, vascular stalk due to the obliteration of the lumen, which connected the fœtal gut with the vitelline cavity, in the early stages of development.

There is also a reduction in the extent of the cavity of the allantoic vesicle though the allantoic mesenchyme and the fœtal mesenchyme and vessels have spread over the whole of the inner surface of the placental cup.

The cytotrophoblastic villi have extended almost to the margin of the placenta, where there occurs a thin layer of syncytiotrophoblast separating the cytotrophoblast from the decidua. The placenta, therefore, appears to be made up of cytotrophoblastic cords which surround lacunæ containing maternal blood, and these cords themselves are surrounded by the allantoic

mesenchyme (Plate XVIII, Fig. 17). In the stage representing the later limb-bud stage the cytotrophoblastic cords have formed a network due to the ramifications of the cords seen in the previous stage, and the meshes of the network are filled by the allantoic mesenchyme and fœtal vessels (Plate XVIII, Fig.18). The threads of the net are formed by the branching, interlacing and interconnected cords of the cytotrophoblast surrounding a thin cytoplasmic membrane (the remnant of the syncytiotrophoblast) and maternal blood.

(*e*) *Stage of Definitive Placenta.*—Parturition takes place about the last week of June or first week of July (Gopalakrishna, 1947). The stage I am describing at present belongs to a specimen collected on the 7th June. The embryo is nearly completely developed. This is further elucidated by the fact that the fœtal erythrocytes have become enucleate.

The yolk-sac at this stage is reduced very much and the cavity of the yolk-sac is almost obliterated—only a little streak-like slit occurring between the bilaminar omphalopleure and the splanchnopleure. Mossman's (1937) figure 'C' on Plate 9 indicates that in Vespertilionid bats the bilaminar omphalopleure shows the presence of an opening communicating the cavity of the yolk-sac with the uterine lumen on the mesometrial side. Such a communication does not exist in *Scotophilus wroughtoni.* I cannot also confirm the observation of van der Sprenkel (1932) of the transformation of the yolk-sac into a gland of internal secretion. I cannot say if this change comes about towards parturition.

The histological structure of the placenta has undergone marked changes. The cytotrophoblastic cords now appear to be made up of rows of flattened nuclei placed in a sheet of cytoplasm because the cell outlines have disappeared (Plate XIX, Fig. 19). The trophoblast thus appears to be made up of a complicated network of labyrinthine columns of trophoblast enclosing maternal blood, and the spaces being pervaded by allantoic mesenchyme and fœtal blood vessels. The fœtal erythrocytes are enucleate and they can be recognised only by their situation. The fœtal and the maternal bloods are separated from each other by (i) the endothelium of the fœtal vessels, (ii) the allantoic mesenchyme, and (iii) a thin layer of trophoblastic syncytium containing flattened nuclei. All these layers are fœtal in origin, there being no remnant of any maternal tissue between the fœtal tissue and the maternal blood. Thus at term the placenta is of the labyrinthine hæmochorialis type as in the case of most of the insectivorous bats so far described.

4. General Conclusions

I have in my collection the developmental stages of two other species of insectivorous bats belonging to two different families—*Taphozoas sp.*

(Emballonuridæ) and *Lyroderma lyra lyra* (Megadermatidæ). I intend to describe in a later paper, an account of the placentation of these, and to give a comparative account of the placentation in these three families of Michrochiroptera-Vespertilionidæ, Emballounuridæ and Megadermatidæ. I shall, therefore, offer here a few remarks to explain the salient features of the placentation in *Scotophilus wroughtoni.*

Towards the end of implantation the trophoblast of the blastocyst has eroded into the uterine tissue on the antimesometrial and the lateral sides. The trophoblast can be recognised into the syncytiotrophoblast and the cytotrophoblast. The syncytiotrophoblast arises by a proliferation of the original primitive trophoblast (of the blastocyst). The cytotrophoblast is the basal trophoblast underlying the syncytio trophoblast and having distinct cell boundaries. A decidua capsularis, as has been described in some of the insectivorous bats belonging to the family Phyllostomidæ, such as *Glossophaga soricina* (Hamlett, 1934 & 1935) and *Artibeus jamaicensis parvipes* (Wislocki and Fawcett, 1941), does not occur in *Scotophilus wroughtoni.*

As in the case of all the species of insectivorous bats so far described, two types of placenta can be observed in *Scotophilus wroughtoni* also. These are functional at two different periods of gestation—a yolk-sac placenta which attains great functional importance during the early stages of fœtal life, and a chorio-allantoic placenta which develops later and continues to be functional till term. Developmentally these two types of placenta may overlap, because at certain stages the chorio-vitelline placenta and the chorio-allantoic placenta are both present. But as the chorio-allantoic placenta becomes more and more elaborate the chorio-vitelline placenta diminishes in functional importance. Actually there is a progressive replacement of the chorio-vitelline placenta by the chorio-allantoic placenta.

A. *Yolk-sac Placentation*

(*a*) *Morphogenesis of the Yolk-sac.*—During the course of the development the yolk-sac undergoes a number of important morphological changes, and each phase is characterised by a number of histological changes. All these changes, which occur during successive periods of fœtal life, start from the embryonic segment of the yolk-sac and progressively pass on to the abembryonic pole. Thus at any particular stage in development there may be differences in the histological details in the different regions of the yolk-sac. The only region of the yolk-sac, which does not undergo much change, is the bilaminar omphalopleure at the abembryonic region, which remains more or less unaltered till term.

The yolk-sac which, at the end of implantation, was lined by the trophoblast and the underlying entoderm (Fig. 1) becomes trilaminar due to

the extension of the mesoderm between these two layers, beginning from the embryonic segment (Figs. 3 & 10). The mesoblast extends up to the margin of the placental cup. At first there is only the mesoderm occurring between the trophoblast and the entoderm, but soon angioblasts make their appearance and the non-vascular trilaminar omphalopleure becomes vascular, starting from the embryonic segment and progressively extending to the placental lip (Figs. 3, 7, 9 & 12). Thus the non-vascular yolk-sac placenta becomes the vascular yolk-sac placenta. Finally, due to the extension of the exocœlom, the vascular trilaminar omphalopleure splits up into an outer somatopleure and an inner splanchnopleure (Figs. 3, 10 & 12). The somatopleure underlies the placental trophoblast while the splanchnopleure carrying the vitelline vessels covers the vitelline entoderm. The exocœlom, therefore, as it extends from the embryonic segment, destroys the vascular yolk-sac placenta from that region. Further change in the gross morphology of the yolk-sac is the result of the invagination of the vascular roof of the yolk-sac over the bilaminar omphalopleure due to the expansion of the amniotic cavity and the exocœlom. This results in the reduction of the extent of the yolk-sac cavity (Figs. 12 & 14). In advanced conceptuses the vitelline cavity occurs as a small space bounded by the bilaminar omphalopleure on the mesometrial side and the vascular splanchnopleure on the antimesometrial side.

(*b*) *The Non-vascular Yolk-sac Placenta.*—The yolk-sac wall attains placental relationship with the uterus at the end of implantation. At first the omphalopleure is throughout bilaminar being formed by the trophoblast and the entoderm. Only on the mesometrial side the yolk-sac wall does not contact the uterine tissue and remains as such till term. The placental trophoblast can be recognised into two regions—the superficial cytotrophoblast which is distinctly cellular, and made up of columnar cells, and the penetrating syncytiotrophoblast which does not show any cell boundaries and progressively penetrates deeper into the uterine endometrium (Figs. 5 & 9). The syncytiotrophoblast is derived by the rapid proliferation of the cytotrophoblast, so that a clear boundary between the two cannot be made out. As the syncytiotrophoblast penetrates more and more into the endometrium, the endothelium of the maternal capillaries is finally lost. Large trophoblastic lacunæ containing maternal blood appear.

The yolk-sac entoderm is made up of a single layer of fusiform cells (Figs. 5 & 16).

(*c*) *The Vascular Yolk-sac Placenta.*—As the mesoblast extends between the trophoblast and the entoderm it converts the bilaminar omphalopleure into a trilaminar omphalopleure. The mesoblast extends from the embryo

up to the lip of the placental cup. Angioblastic tissue soon appears in the mesoblast, and the area vasculosa extends right up to the lip of the placental cup. Thus the vascular chorio-vitelline placenta is produced, which is of some physiological importance till the chorio-allantoic placenta is formed.

Histologically the chorio-vitelline placenta shows the separation of the fœtal and maternal bloods by the fœtal endothelium, the yolk-sac mesenchyme, the cytotrophoblast, and the syncytiotrophoblast. The maternal endothelium will have been lost by the time the yolk-sac wall becomes vascular.

As a result of the extension of the exocœlom and the amniotic cavity the vascular splanchnopleure of the yolk-sac is separated from the somatopleure and the chorio-vitelline placenta becomes obliterated. Further the increased functional importance of the chorio-allantoic placenta reduces the significance of the yolk-sac as a placental structure. Thus the yolk-sac placenta is functional for a short period in embryonic life.

The yolk-sac placenta of *Scotophilus wroughtoni* therefore resembles the yolk-sac placenta of *Vesperugo leisleri* (Ramaswamy, 1933), and *Myotis lucifugus lucifugus* (Wimsatt, 1945). As in these cases the distal wall of the yolk-sac remains distinctly bilaminar. There is nothing comparable to the 'Inverted yolk-sac placenta'. The distal wall of the yolk-sac does not get perforated, as is apparent from Mossman's (1937) figure of the yolk-sac of the Vespertilionid bat (Plate 9, Fig. C. Mossman, 1937). So the yolk-sac cavity does not become continous with the uterine lumen.

B. *Chorio-allantoic Placentation*

I have given a fairly detailed account of the penetration of the endometrium by the trophoblast and the formation of the trophoblastic lacunæ in my earlier paper (1949 *b*). Thus, at the end of implantation, the chorionic placenta will have been established and this is already of a hæmochorial complexity in most places. The further structural changes in the placenta consist in the vascularisation of this chorionic complex by the fœtal vessels through the allantois resulting in the formation of a labyrinthine hæmochorial placenta.

As development proceeds the thickness of the placenta increases due to the further invasion of the uterine endometrium by the syncytiotrophoblast. Secondly, the shape of the placenta becomes changed from the original cup-shaped condition to a hollow saucer-shaped condition. More than the gross morphology, the histological structure of the placenta undergoes marked changes. As the allantois grows across the exocœlom towards the placenta, it carries with it fœtal vessels and the mesoderm. The latter gradually spreads

out towards the margin of the placental cup carrying along with it the allantoic vessels. Simultaneously with the spreading of the mesoderm on the base of the placenta, the cytotrophoblastic layer becomes active and begins to send in, by proliferation, cords of cells into the syncytiotrophoblast (Figs. 4, 7, 12 & 14). These cords as they penetrate the syncytiotrophoblast towards the decidual region, produce large excavations in the syncytiotrophoblast, into which the allantoic mesenchyme and the fœtal blood vessels enter. The cytotrophoblastic cords at first occur as columns of cytotrophoblastic cells surrounding the syncytiotrophoblast and the lacunæ (Fig. 17), but later by the formation of lateral branches, the whole placenta appears like a network of tubes (Fig. 18), and the meshes are occupied by the allantoic mesenchyme and the fœtal vessels. The threads of the net are tubular and formed by a thin layer of syncytiotrophoblast supported by a layer of cytotrophoblast. The lumina of the tubes contain maternal blood. The cytotrophoblast does not come into direct contact with the maternal blood.

The further reorganisation of the placenta consists in the elimination of the cytotrophoblastic cords which occurs as a layer between the allantoic mesenchyme and the syncytiotrophoblast. The cells of the cytotrophoblast gradually lose their cellular boundaries, and in later stages only flattened darkly staining nuclei occur in rows forming a wall to the fœtal mesenchymal tissue. Thus at final stages the placenta is made up of ramifying tubules of trophoblast occurring in the allantoic mesenchyme and fœtal blood vessels. The maternal blood occurs inside the trophoblastic tubules.

Thus when fully formed, the placenta is labyrinthine and hæmochorial and the tissues that separate fœtal and maternal bloods are all fœtal in origin—the maternal endothelium and the endometrial tissue being destroyed at a very early stage of development.

5. Summary

1. The yolk-sac which is disposed towards the mesometrial side establishes placental relationship with the uterus. The yolk-sac placenta is at first non-vascular and later becomes vascular.

2. As development proceeds the extension of the exocœlom and the expansion of the amniotic cavity results not only in the obliteration of the yolk-sac placenta, but, in the invagination of the yolk-sac roof over the distal bilaminar omphalopleure.

3. The allantois is disposed towards the antimesometrial side. The allantois gradually spreads towards the lateral sides below the placenta and progressively replaces the yolk-sac placenta.

4. The changes involved in the formation of the definitive labyrinthine placenta are: (i) The penetration of the syncytiotrophoblast by the trophoblastic chords, (ii) the invasion of the cavities thus formed by the allantoic mesenchyme and fœtal vessels, (iii) the loss of the cell boundaries in the cytotrophoblastic cords, which therefore occurs as a network of cytoplasm with flattened nuclei embedded in rows.

5. The final placenta is therefore of the labyrinthine hæmochorial type.

6. Acknowledgement

I am deeply indebted to Prof. M. A. Moghe, Head of the Department of Zoology, College of Science, Nagpur, for his valuable guidance throughout this work and for having allowed me the use of his rich personal library.

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(*Abbreviations used.—All. d.*, Allantoic diverticulum; *All. ve.*, Allantoic vesicle; *Am.*, Amnion; *Am. c.*, Amniotic cavity; *Bi. om.*, Bilaminar omphalopleure; *Exo.*, Exocœlom; *F. V.*, Fœtal blood vessel; *M. Bl.*, Maternal blood vessel; *Pl.*, Placenta; *Spl.*, Yolk-sac splanchnopleure; *Y. S.*, Yolk-sac cavity.)

N.B.—The arrow points towards the mesometrial side wherever it is drawn. All figures are photomicrographs, and magnifications are approximate.

Explanation of Plates

Plate XIII

Fig. 1. The Implanted blastocyst. The amniotic cavity is formed and the trophoblast has penetrated the uterine endometrium on the antimesometrial side. On the mesometrial side it is free so that a part of the uterine lumen persists, and large mouthed uterine glands are seen to open into this part of the uterine lumen. × Circa 25.

Fig. 2. A part of the antimesometrical wall of the uterus of the same specimen as seen in the previous figure enlarged (a part of the rectangle in Fig. 1). Note the trophoblast penetrating into the endometrium. The large lacunæ are filled with maternal blood. Note the villi-like cords of the cytotrophoblast entering the endometrium, and in deeper layers it becomes syncytial. × Circa 135.

Fig. 3. Stage of early neural groove formation. The mesoderm has differentiated and has extended up to about the middle of the lateral wall of the yolk-sac. × Circa 35.

Plate XIV

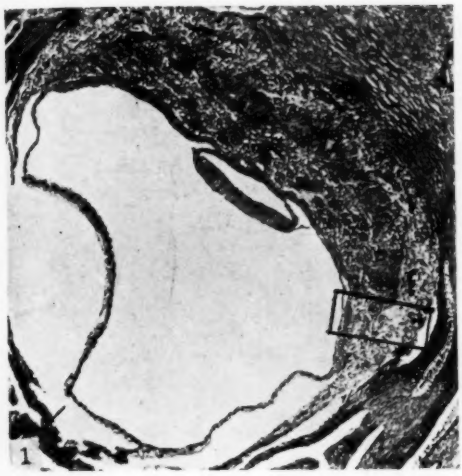
Fig. 4. A part of the blastocyst in Fig. 3 (part of the rectangle near the embryonic disc), magnified. The endoderm, made up of a single layer of flattened cells, can be clearly seen underlying the mesoderm. Note the penetration of the trophoblast into the endometrium and the formation of large lacuanæ containing maternal blood and surrounded by the trophoblast. Deeper to the layer of the syncytiotrophoblast, the uterine endometrium shows a layer of loose tissue full of spaces. × Circa 290.

Fig. 5. Lower part of the lateral wall of the yolk-sac in Fig. 3 (part of the lower rectangle enlarged). The mesoderm does not occur in this region. A part of the free bilaminar omphalopleure is also included in the figure. × Circa 170.

Fig. 6. Later neural groove stage. Only the embryonic disc is shown to indicate the formation of the cœlom in the mesoderm which occurs on the two sides of the middle line. × Circa 150.

Plate XV

Fig. 7. A part of the lateral wall of the yolk-sac of the specimen shown in Fig. 6. Note the extensive penetration of the trophoblast into the uterine endometrium, and the complete loss of the endothelium of the maternal vessels. The trophoblast is syncytial all round, except the basal cytotrophoblast where cell boundaries are distinct. Angioblasts can be seen below the layer of the cytotrophoblast. A large lacuna containing maternal blood is included in the figure. × Circa 275.



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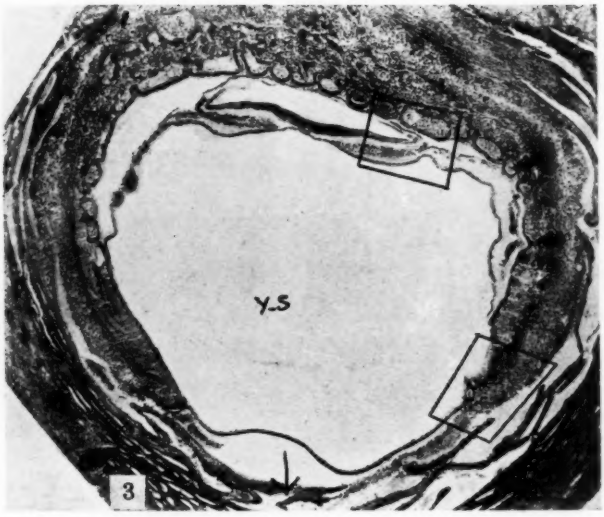

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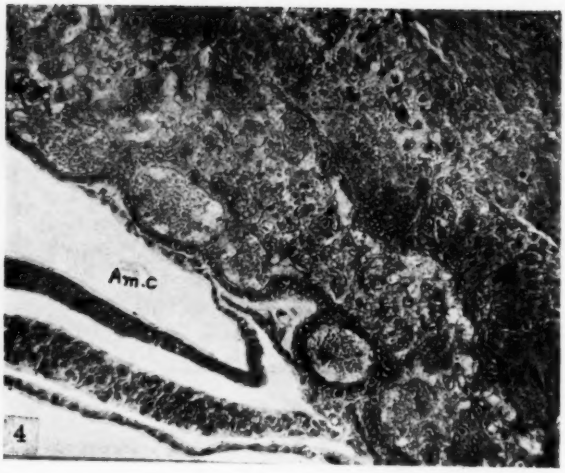
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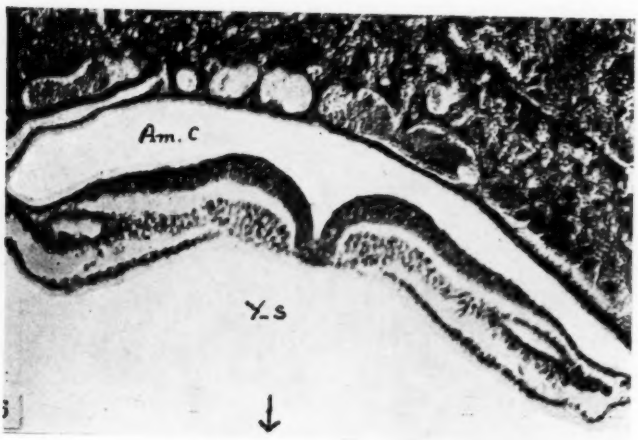
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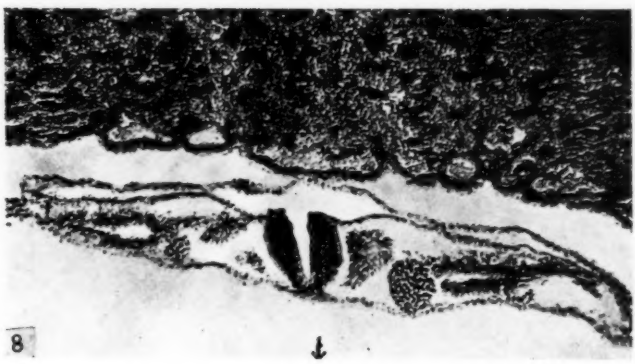
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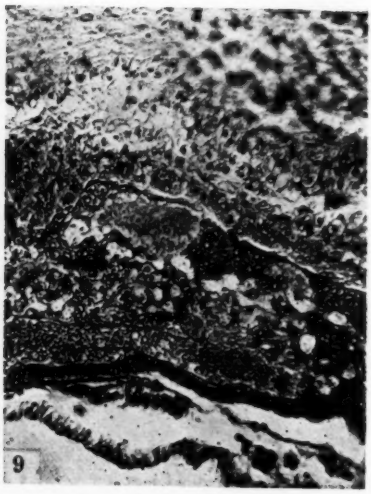

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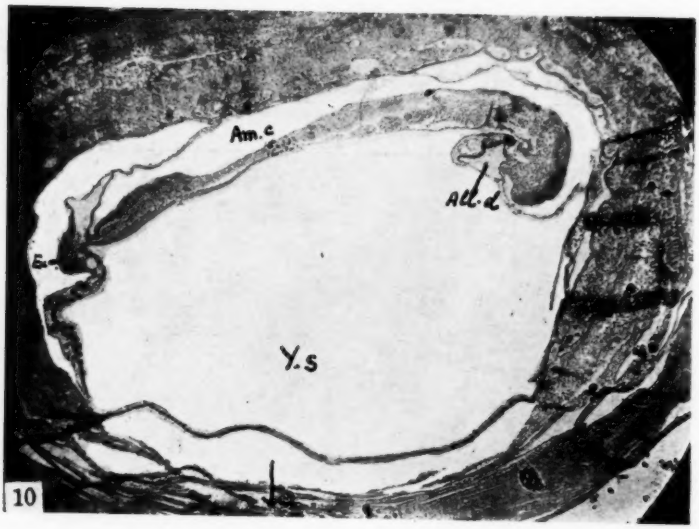

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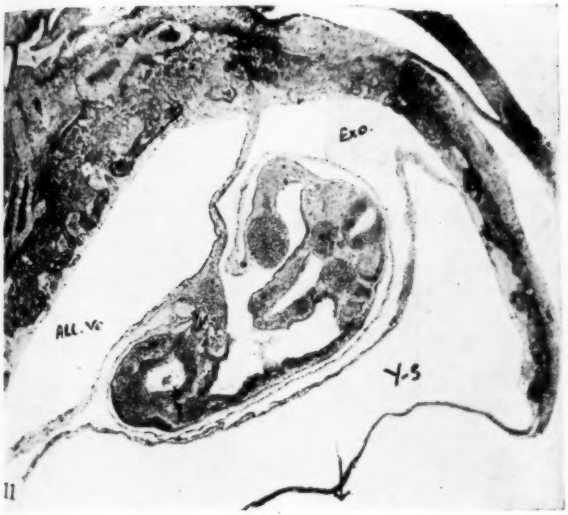
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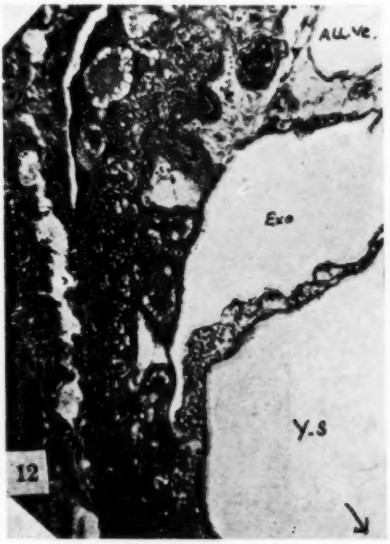
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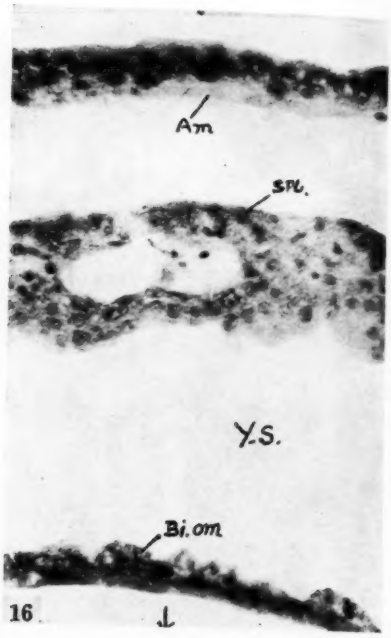

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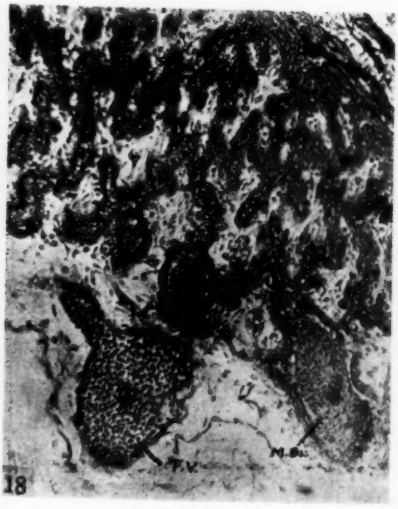

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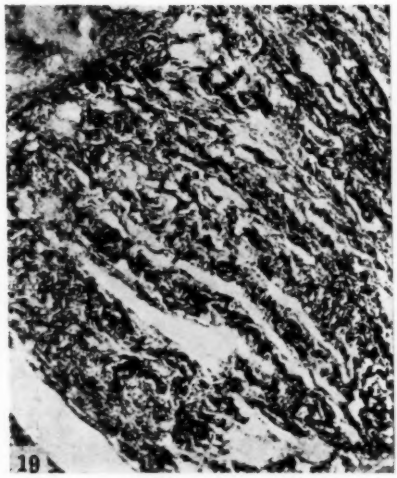
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FIG. 8. Late stage in the formation of the neural groove. The mesoderm shows the formation of the myotomes, and the fœtal vessels are beginning to be formed. × Circa 125.

PLATE XVI

FIG. 9. A part of the lateral wall of the uterus of the specimen shown in Fig. 8. The yolk-sac wall shows the presence of fœtal blood vessels. The syncytiotrophoblast has penetrated still further. Cell boundaries are very clear in the basal cytotrophoblast. Below the placental layer the uterine endometrium shows a layer full of intercellular spaces. × Circa 185.

FIG. 10. Longitudinal vertical section of an embryo with closed neural groove. Note the vascularisation of the yolk-sac up to the placental lip. The allantoic rudiment occurs as a slight diverticulum on the posterior part of the embryo (towards the right hand side of the figure). × Circa 35.

PLATE XVII

FIG. 11. Embryo showing the allantois carrying foetal vessels to the placenta on the antimesometrial side. The exocœlom has destroyed the yolk-sac placenta for some distance in the embryonic segment. So the yolk-sac placenta occurs only on the lateral sides. × Circa 35.

FIG. 12. Part of the gestation sac of the specimen shown in Fig. 11, showing the yolk-sac placenta and a part of the allantoic placenta. Note the presence of the cytotrophoblastic chords penetrating the syncytiotrophoblast, and the penetration of the allantoic mesoderm and fœtal vessels into the excavations so formed by the cytotrophoblast. The deciduo-syncytial junction is quite clear. The zone of separation of the parturient is already foreseen in this stage by the formation of a cleft-like space which occurs all round the placental region. × Circa 140.

FIG. 13. Part of the placenta of a specimen described in Stage II of the text. The syncytiotrophoblast is invaded by the cytotrophoblastic cords, the allantoic mesenchyme and the allantoic vessels. × Circa 160.

FIG. 14. Part of the uterus containing an embryo wherein the yolk-sac placenta is completely destroyed as a result of the extension of the exocœlom. The cavity of the yolk-sac is reduced to a slit-like space because of the expansion of the amniotic cavity. The bilaminar omphalopleure is persistent. × Circa 35.

PLATE XVIII

FIG. 15. Part of the placenta of the specimen shown in Fig. 14. Note the extensive penetration of the allantoic mesenchyme and the fœtal vessels into the placenta. × Circa 130.

FIG. 16. A part of the amnion and a part of the yolk-sac are shown in the figure. The roof of the now reduced yolk-sac carries the vitelline vessels. × Circa 240.

FIG. 17. A part of the placenta in the early limb-bud stage. The placenta is made up of tubules of cytotrophoblast surrounding a thin layer of syncytiotrophoblast; the tubules contain maternal blood corpuscles. The tubules are separated by allantoic mesenchyme containing fœtal vessels. × Circa 150.

FIG. 18. Placenta in late limb-bud stage. The placenta has become typically labryinthine and hæmochorial. The spaces of the labyrinth are filled with allantoic mesenchyme and fœtal vessels. The fœtal erythrocytes still have nuclei. × Circa 105.

PLATE XIX

FIG. 19. Placenta in late stage from a specimen collected on 7th June. The placenta is made up of a complicated trophoblastic network. The trophoblast has become syncytial. The fœtal vessels are found between the trophoblastic cords of the net. The fœtal erythrocytes have lost their nuclei. × Circa 130.

STUDIES ON LENGTH FREQUENCY OF THE OIL SARDINE, *SARDINELLA LONGICEPS*, CUV. & VAL., AND ON CERTAIN FACTORS INFLUENCING THEIR APPEARANCE ON THE CALICUT COAST OF MADRAS PRESIDENCY*

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1. Introduction

The fluctuations in the fishery of the Oil Sardine—*Sardinella longiceps*, Cuv. and Val. on the West Coast of the Madras Presidency are so great that they render the fishery commercially undependable. "The Oil Sardine is very capricious as to its arrival and departure, thus in 1855–56 from the port of Cochin, only 45 cwt. of fish oil was exported, the next year nearly 181 cwts. but in 1857–58, 68,499 cwts., which augmented in the succeeding year to 102,924 cwts. and the year after to 133,143 cwts.", so observed Day as early as 1865. Hornell (1910) in presenting the statistics for the period 1896 to 1907, recorded that "in 1907 Sardines were more abundant than they had been since 1896". Nicholson (1922) and Hornell (1924) indicated the success of the Oil Sardine fishery only in 1912–13, 1919–20 and 1922–23. The records of fish statistics of the West Coast of the Madras Presidency from 1925–26 onwards show that the landings of the Oil Sardine exceeded 1,000,000 maunds† only in 1925–26 and 1933–34, the catches were less than 50,000 maunds in 1928–29, 1932–33, 1934–35, 1935–36 and 1942–43. The maximum landing during the period 1925–26 to 1942–43 was 1,923,593 maunds, in 1933–34.

Hornell (1924) wrote that the preliminary investigations on the life history of the Oil Sardine, *Sardinella longiceps* Cuv. and Val., made in 1908 "showed how essential to the future development of these fisheries is a sound knowledge of the factors controlling and influencing both the seasonal migrations and extreme fluctuations frequently seen in the annual abundance" of the Oil Sardine. The investigations conducted in 1922 contributed some knowledge on the food, the seasonal growth, the age, sexual maturity, etc.

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† 28 Maunds = 1 ton.

of the Oil Sardine. Hornell in summarizing the observations on Oil Sardines in 1923, indicated the possible factors influencing the migration of the young and adult sardines. Devanesen (1943), following the lines of investigation initiated by Hornell, was able to confirm certain of the latter's observations in addition to providing further information on the subject. He based his investigations on three hypotheses; they were (1) the effect of adverse hydrographical conditions on the shoreward migration of the Oil Sardine, (2) the temporary abandonment of this coast by the sardines and (3) the probable over-fishing of shoals appearing on the coasts of the Malabar and South Kanara districts. In his report he recommended that 'the question of age and kindred researches, such as the age composition of oil sardine shoals may be pursued', in addition to a few other lines of investigation. The present paper is a continuation of the investigations and is based on the study of the length frequencies and age compositions of the Oil Sardine and of the effect of certain hydrological and food factors on the spawning, survival and the movements of the Oil Sardine on the Malabar coast, during the years 1936–37 to 1942–43 when the Oil Sardine fishery was generally poor. An attempt has been made to determine the age composition of the Sardines in different years, the year classes contributing to the fishery and also to present the possible correlation between surface temperature and specific gravity of sea water and food organisms with the appearance of the Oil Sardines along the coast.

2. Materials and Methods

Records of examintion of Oil Sardine samples collected at random from fishing boats on the West Coast are maintained regularly at the West Hill Biological Station with details of locality, date, length, condition of gonads, food, etc. The data collected and recorded in the fish registers of the Marine Biological Station, West Hill, during the seven year period 1936–37 to 1942–43 are compiled and interpreted to show clearly the entrance into, the progression through, and the eventual disappearance from the fishery of the various size groups of the Oil Sardine in the different years under study. The length frequencies are tabulated for the seven seasons, 1936–37 to 1942–43 (*vide* Appendix I). The sardines, under study, were caught by fishermen in (1) two types of boat seines, *viz.*, " Painthu vala " with a mesh of 15 mm. (diagonal) and " Mathikolli vala," having a mesh of 18 mm. (diagonal) and (2) one type of gilling net, called " Mathichala vala " having a mesh ranging in size from 35 mm. to 45 mm. (diagonal).* The measurements of Oil Sardine caught in the inshore areas by the fishermen in the different months of the season when they are caught in the region between

Calicut and Quilandy, a distance of 15 miles, are only taken into consideration in the present study. The fish statistics are collected from the various fish curing yards on the West Coast and the different statements compiled at West Hill form the basis for the statistics given in this paper. At West Hill, meteorological and hydrological data including atmospheric temperature, humidity, rainfall, surface temperature and specific gravity of sea water opposite the Biological Station are recorded daily in the morning. The hydrographical data referred to in the paper are taken from the records thus maintained at the Station.

The length of fish mentioned in the paper is the total length from the tip of snout to the tip of the caudal fin of the Oil Sardine. The temperature of the sea water was taken by P. T. R. surface thermometer. The specific gravity was recorded by Seewasser Araometer.

3. Length Frequency Studies

The data of length frequencies referred to above include the sizes in the commercial catches in each fishing season, irrespective of the fishing methods and the extent of the season. As the number of samples and the number of fish measured varied greatly from year to year, the numbers of each length are expressed in percentages of the total so as to make the length frequencies directly comparable for each season. The frequencies expressed are for both sexes together.

The frequencies of each length unit for an average season are obtained by taking the average percentage of the length frequency for the seven seasons. The average frequency is then subtracted from each annual percentage frequency to obtain the deviation.

Size groups

Fig. 1 represents the length frequency curves of Oil Sardine—*Sardinella longiceps* Cuv. and Val. for the seven seasons, 1936–37 to 1942–43. These curves show how the Oil Sardine fishery of each season consists of fish of different size groups constituted by the different generations in the population. The entrance into, the progression through and the eventual disappearance from the fishery of these different size groups are indicated here; and a close study of the histograms enables one to understand the probable effects on the fisheries caused by fishing, of any particular size group or groups through this period.

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* *Vide* Hornell, J., "The fishing methods of the Madras Presidency, Madras," *Fish. Bull.*, 1938, **27**, 51–64.

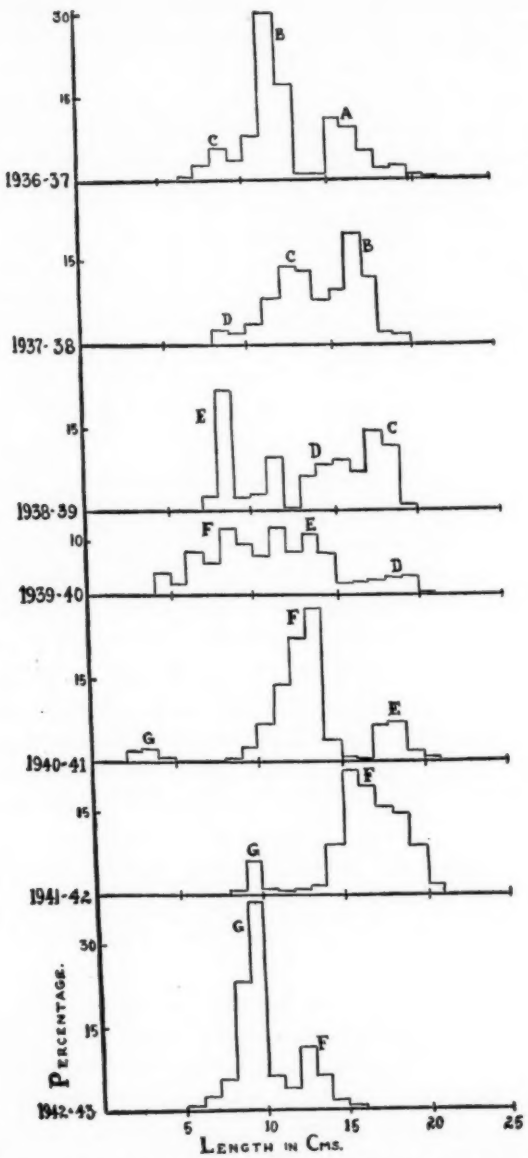

Fig. 1. Histogram showing the length frequencies of Oil Sardine—*Sardinella longiceps* Cuv. and Val. for the seasons 1936–37 to 1942–43.

A, B, C, D, E, F and G indicate different groups of sizes and age, comprising the fishery.

In the season of 1936–37 the Oil Sardine fishery comprised of 3 size groups, A, B and C. Group A represented sardines measuring 16·0 cms. in length, group B, 12·0 cms. and group C, 9·0 cms. Group A was not traceable in the following year and had naturally been in the last phase. B shows a marked dominance and constituted a greater part of the year's fishery. Group C was probably entering the fishery for the first time. The contributions of A and C to the year's fishery being very small, the season's fishery depended entirely on group B.

During the season 1937–38 groups B and C contributed to the fishery. B had maintained its dominance and C also had been abundant in the year. Group A of the previous year had practically disappeared from the fishery. Group B had shown an increase in length from 12·0 cm. in 1936–37 to 17·0 cm. in 1937–38 registering an average increase of 5·0 cm. The length of group C had increased by 4·5 cm., from 9·0 cm. to 13·5 cm.

In 1938–39 three groups constituted the fishery. Besides groups B and C of the previous season, two new groups D and E had contributed to the fishery. D entered as a minor group in 1937–38 and E was dominant in the first season of its appearance. C, though not dominant, had continued to contribute to the fishery to a fairly good extent. Group C had increased by about 5·0 cms. between the two seasons. Group D measured 14·0 to 16·0 cms. and E, 9·0 to 12·0 cms. in length.

In the season 1939–40, group D had begun to disappear from the fishery. E and F had contributed equally to this year's fishery, their average length being 12·0 to 15·0 cms. and 9·0 to 12·0 cms. respectively. Two other minor size groups of fish smaller than E were observed in the fishery during the season but they had disappeared from the fishery very early.

In 1940–41, group F had dominated the fishery and the average size had increased in length by 4·0 cms. E had increased in length by about 5·0 cms. during the last year. Besides E and F, a new group G of very small sardines, 3·5 cms. in length had made its appearance in the fishery during the season.

In 1941–42, groups E and F together had contributed to the success of the fishery. G had persisted as a minor group and had increased from a length of 3·5 cm. to 10·0 cms., a growth of 6·5 cms. in the period.

In 1942–43, E had disappeared. Group G had attained great dominance and F had been relegated to a minor position. The fishery of the year depended entirely on group G, which had grown by 5·0 cms., from 10·0 cms. in the previous year to 15·0 cms. in the present year. Group F recorded an increase of about 4·0 cms. in this year.

Age groups—(Table I and Fig. 2)

From the study of the length frequency and size groups, the composition of age groups is indicated. Of the seven groups A, B, C, D, E, F and G, which had passed through the fishery during the period 1936–37 to 1942–43, group G showed the smallest sized fish at the time of entrance into the fishery, the length being only 3·5 cms. This group had just entered the fishery in 1940–41, in its first year, the age being less than one year. This group had registered an increase in length of 6·5 cms. in the year, measuring 10·0 cms. and 5·0 cms. in the second year, measuring 15·0 cms. in length.

At the time of entrance F measured 9·0 to 10·0 cms. And the group had been in its first year of life. In its second year of growth, it had increased in length by 4·0 cms; thereafter in its 3rd and 4th years, it showed an increase of growth of 2·0 cms. and 4·0 cms. each year. In 1943 in its fourth year of life the group measured 20·0 cms.

Group E entered the fishery in 1938–39. E measured 9·0 to 12·0 cms. at the time in its first year of life. In the second year E measured 15·0 cms. and in its third year E showed an increase to 18·0 to 19·0 cms. and in the fourth year to 20·0 to 21·0 cms.

Group D measured 9·0 cms. at its entrance into the fishery in 1937–38 and 14·0 cms. to 16·0 cms. in its second year and 19·0 to 20·0 cms. in its third year, registering an average increase of 5·0 to 7·0 cms. in the second and 5·0 to 6·0 cms. in the third year.

TABLE I

Maximum size of the groups in cms. of the Oil Sardine in the different years

	A	B	C	D	E	F	G
1st year ..	..	..	9	9	12	10	10
2nd year ..	..	13·0	14	16	15	14	15
3rd year ..	18·0	18·0	19	20	19	16	..
4th year ..	..	..	..	..	21	20	..

Group C measured 9·0 cms. in 1936–37 and in its first year this group had entered the fishery. In the next season the group measured 13·0 to 14·0 cms. having increased by 4·0 to 5·0 cms. in its second year. The length of the group was 18·0 to 19·0 cms. in its third year.

Group B entered the fishery in 1936–37 ranging in length between 11·0 and 13·0 cms. which was the length in other groups, early in their second year. So the presumption is that B in 1936–37 was in its second year of life. In its third year B measured 17·0 to 18·0 cms. having registered an increase of 5·0 cms.

Group A in 1936–37 measured 15·0 to 18·0 cms. This length was recorded for C and D in their second and third year. This group must be in the beginning of the third year in the fishing season of 1936–37, and it is possible that this group consisted of those spawned at the beginning and end of the season in 1934.

Size progression of the groups

Fig. 2 represents the annual size progression of the different groups in the fishery. The size progression according to the ages of the different

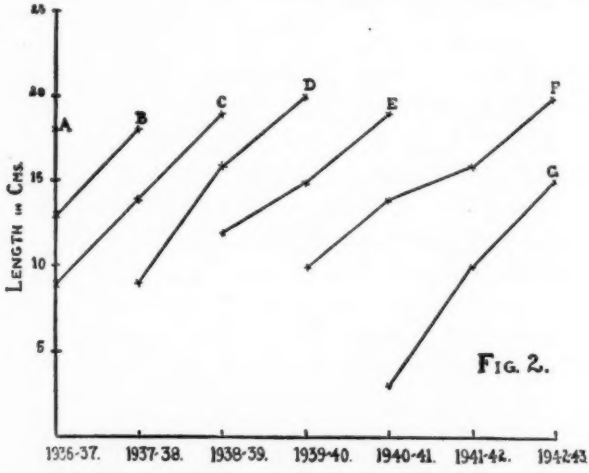

FIG. 2.

Fig. 2. Graph showing the length of the different age-groups in the years from 1936–37 to 1942–43.

A, B, C, D, E, F and G indicate the different size and age groups.

groups is indicated in Table II. E represented the size for the one year old group and F came into the fishery in the last months of the year and it was in its first year of life and had not completed one year. C and D showed a length of 9 cms. and F 10·0 cms. and B, D and E 12·0 cms. in their first year. In the second year B, C, D and E were 17·0 cms., 13·5 cms., 14·0 to 16·0 cms. and 12·0 to 15·0 cms., respectively. F measured 13·0 to 14·0 cms. in length. In the third year groups D, E and F showed a length

of 19·0 to 20·0 cms., 18·0 to 19·0 cms., and 16·0 cms., respectively, and C 18·0 to 19·0 cms. In the fourth year, E measured 20·0 to 21·0 cms. and F 17·0 to 20·0 cms. The average rate of growth of these age groups at the end of each year of their life is as follows:—

TABLE II

Size progression of the groups

Period	Average length in cms.	Rate of increase in cms.
1st year ..	10·0	..
2nd year ..	14·5	4·5
3rd year ..	18·3	3·8
4th year ..	20·5	2·2

Seasonal deviations of the length frequency from average frequency of the period

The curves in Fig. 3 indicate the deviation of each seasonal frequency curve from the average frequency curve for the seven seasons. In addition to the groups A, B, C, D, E, F and G, certain size groups characterised by scarcity of fish were present. These deficient groups referred to as *a*, *b*, *c*, *d*, *e*, *f* and *g* showed the same type of size progression as the dominant groups.

A scarcity of sardines of size class just greater than that of dominant group A as depicted by depression '*a*' was evident during the first season, 1936–37; '*a*' was not evident in the following year.

The deficiency of sardines between size groups A and B was expressed in depression '*b*' which was manifest in a marked degree during the first season 1936–37 and was noticeable in the following year also.

Depression '*c*' revealed the scarcity of fish immediately smaller in size than group B. '*c*' was first evident in 1936–37 and was traceable through the succeeding two years.

Deficient group '*d*' was evident in the fishery in 1937–38 and persisted during the next two seasons. This group represented sardines of size immediately smaller than C. Depression '*e*' became manifest during the season 1938–39 and continued to influence the fishery during the following two seasons. '*f*' of size class immediately below E and '*g*' became

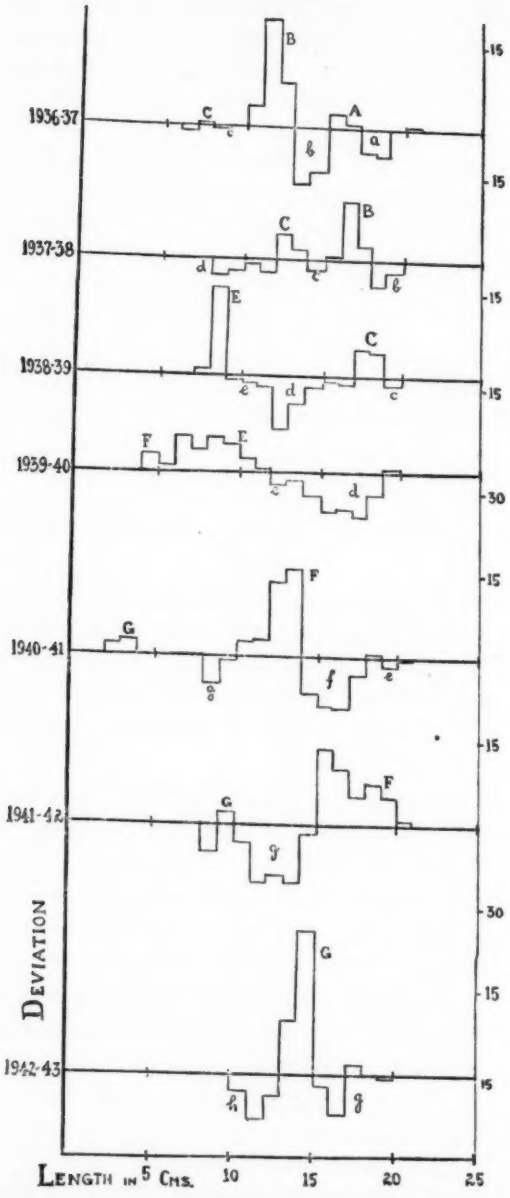

Fig. 3. Histogram showing the deviation of each seasonal frequency from the average frequency of the dominant groups. This also indicates the presence of deficient groups.

evident in 1940–41 and '*g*' gained prominence in 1941–42. The group '*g*' was evident in the following season also. '*h*' appeared in the last season 1942–43, denoting the deficiency of sardines of size smaller than G.

Size groups and depletion

It is known that the Oil Sardine of Malabar attains maturity when it grows to a length of 15 cms. (Devanesen, 1943, p. 13). Any indiscriminate fishing of immature sardines of sizes below 15 cms. results in a corresponding deficiency of spawners in the succeeding years accounting partly for dwindling of the fishery.

In 1936–37, the dominant size group contributing chiefly to the fishery was B, consisting of sardines 12 cms. long (Fig. 1). Group C had been fished to a small extent in 1936–37 and to a much greater extent in 1937–38. In 1938–39, we have two groups of sardines below 15 cm. length, exploited. The dominant group of the season E, was of size 9 cms. only and group D measured 14·0 to 15·0 cms. In the following year 1939–40, groups E and F again comprised of immature sardines. These groups, together with two other groups of size, smaller than 9 cms. contributed to the fishery of the season. The immature sardines had been the mainstay of the fishery during the period. The persistent exploitation of immature sardines during the preceding years has possibly resulted in a deficiency of mature sardines during 1939–40. This deficiency is well marked in the corresponding curve in Fig. 2, where it is represented by two deficient groups '*d*' and '*e*'. This scarcity of mature sardines is bound to have an adverse effect on the fishing in years to follow, unless the conditions for spawning of the small proportion of mature individuals and the survival of the young are favourable. In 1940–41, the fishery depended almost entirely on group F. This group also represented immature sardines, of size 14 cms. During the following season, however, the majority of catches were formed of sardines above 15 cms. length and in the last season 1942–43, the dominant group G measured 15 cms.

Of the seven seasons covered by this study, in the first five seasons, there had been exploitations of immature sardines. The seasons 1941–42, and 1942–43, showed landings of mature sardines, but by this time, the dwindling of Oil Sardine shoals on the Malabar coast had begun to be manifest. The absence of new groups of immature sardines of size immediately below 15 cms. and above the size of group F, in the seasons 1940–41, 1941–42 and 1942–43, is itself a proof of lack of spawners in sufficient numbers in the preceding seasons that would otherwise have given rise to new groups of small-sized sardines, and if the factors influencing a successful survival of the young ones have been favourable.

4. The Contribution of the Different Year Classes to the Fishery

From a study of the length frequencies and size groups of the Oil Sardines of Malabar coast during the period 1936–37 to 1942–43, the life of the Oil Sardine may be said to be between 3 and 4 years. The average length of the Oil Sardine being 10·0 cms., 14·5 cms., 18·3 cms. and 20·5 cms. in their 1st, 2nd, 3rd and 4th years respectively.

In 1936–37 the specimens examined revealed the presence of large number of individuals measuring less than 15·0 cms. and whose gonads are indeterminate and they appeared in large numbers in the months of November and December of 1936 and January of 1937. In specimens measuring less than 20·0 cms. the samples showed equal proportion of sexes; but in the lot measuring more than 20·0 cms. the females were predominant. In June 1937, only specimens measuring more than 15·0 cms. were observed, thus indicating the absence of a good survival class of those spawned in the 1935 season.

In 1937–38 the catches showed a large proportion of the Sardines measuring above 15·1 cms. but below 20·0 cms. in July, August and December 1937 which indicate the contribution made by the year class which was responsible for the fishery of 1936–37 also. In September, October, November and December of 1936, the sizes between 10·1 and 15·0 cms. dominated the samples and they were less than 13·0 cms. and they were sexually indeterminate; the dominance of this group showed a fairly good survival of those spawned late in 1935. The samples showed an equal proportion of sexes in specimens measuring between 15·1 and 20·0 cms.

In 1938–39, there had been a fairly equal representation of both sexes in the two groups of sizes, *viz.*, 10·1 to 15·0 cms. and 15·1 to 20·0 cms. from August 1938 to February 1939. But in September 1939 there was a good representation of Oil sardines measuring less than 10·0 cms. belonging to the groups spawned in 1938.

In September 1939, a large number of Oil Sardines measuring less than 10·0 cms. were met with in the samples, 42% of Oil Sardines measuring less than 10·0 cms., 36% between 10·0 cms. and 15·0 cms. and 19% between 15·1 cms. and 20·0 cms. The Oil Sardines less than 10·0 cms. belonged to the group spawned in 1938.

In September 1940 large number of Oil Sardines measuring 3 to 4 cms. in length were caught; these evidently belonged to the group which spawned early in June of that year and had grown to that size within 4 months. It is likely that there may be two size groups each representing

an individual wave of spawning in any one year in the locality, if the young ones hatched during the extended spawning period from June to November survive and the conditions have been favourable for their survival.

In 1940–41 season Oil Sardines measuring between 10·0 cms. and 15·0 cms. predominated in the samples, indicating the contribution to the fishery of the group spawned in 1938, as specimens whose sexes were not distinguishable, appeared in large numbers in October, November and December of 1940 and January and February of 1941. 20% of the samples measured between 15·1 to 20·0 cm. in length, thus showing the survival of the group which spawned in 1938, to contribute to the fishery in its fourth year. In September 1940 there were small sardines measuring less than 4·0 cms. in length and these belong to those spawned early in June of that year.

From July 1941 to February 1942 the samples examined were dominant in sizes between 15·1 to 20·0 cms. in length and these were contributed by the group spawned in 1939. The sizes above 20·1 cms. were represented in this year.

The absence of young sardines from January 1941 to December 1942 indicates that there was no successful spawning late in 1940, in 1941 and in 1942 and a reference to the tables for that period shows a general reduction in the percentage of representation of the successive size groups leading towards a decline in the Oil Sardine fishery.

Basing on the above data, the year classes which had contributed the fishery in the subsequent years and the quantity of Oil Sardines landed on the coast between Calicut and Quilandy are given in the following table:—

TABLE III

Year class	Season of capture	Quantity of Oil Sardine in maunds
1934 ..	1936–37	1,36,405
1934–35 ..	1937–38	91,037
1935–36 ..	1938–39	6,120
1937–38 ..	1939–40	14,528
1938–40 ..	1940–41	1,42,229
1938 ..	1941–42	17,976
1941 ..	1942–43	3,063

5. FACTORS INFLUENCING SPAWNING AND SURVIVAL OF OIL SARDINES

The fluctuations in abundance of shoaling fishes, independent of those effected by the fishery, are the results of the varying degrees of success of

TABLE IV

Table giving details of the dates of appearance and disappearance of the Oil Sardine, on the West Coast of Madras Presidency

Name of Yards	1936–37		1937–38		1938–39		1939–40		1940–41	
	Date on which they appeared first	Date on which they disappeared	Date on which they appeared first	Date on which they disappeared	Date on which they appeared first	Date on which they disappeared	Date on which they appeared first	Date on which they disappeared	Date on which they appeared first	Date on which they disappeared
Malpe ..	4th Oct.	20 Nov.	9 Oct.	23 Nov.	3 Nov.	20 Jan.	5 Dec.	26 Jan.	6 Sept.	27 Dec.
Udayawar ..	1st Jan.	9 Jan.	22 Oct.	13 Nov.	11 Nov.	25 Feb.	4 Nov.	16 Jan.	29 Aug.	25 Nov.
Madai ..	1st Sept.	7 Feb.	27 June	25 Dec.	6 Oct.	26 Dec.	25 Sept.	15 Feb.	16 Sept.	19 Jan.
Cannanore ..	29 Aug.	21 Jan.	10 Aug.	11 Jan.	12 Nov.	7 Dec.	18 Sept.	30 April	16 Oct.	4 Feb.
Tellicherry ..	15 Aug.	8 Feb.	2 Aug.	7 Jan.	1 Sept.	22 Nov.	16 Sept.	21 Dec.	26 Sept.	25 Jan.
Quilandy ..	27 Aug.	22 March	28 June	9 Jan.	5 Aug.	23 Aug.	22 Sept.	22 Feb.	19 July	7 Feb.
Calicut (North) ..	4 Aug.	2 Feb.	26 Aug.	26 Jan.	27 Aug.	12 Feb.	26 Sept.	15 Feb.	29 July	3 Feb.
Tanur (North) ..	5 Aug.	22 April	21 Aug.	10 Jan.	25 Sept.	19 Feb.	2 Aug.	21 April	16 Oct.	6 Feb.
Ponnani ..	4 Aug.	28 April	29 Aug.	18 Jan.	18 Dec.	15 Feb.	19 Oct.	29 Feb.	17 Sept.	12 Feb.
Kadapuram ..	13 Oct.	14 April	9 Sept.	4 Jan.	17 Jan.	19 Jan.	17 Aug.	4 Feb.	27 July	21 Jan.

the spawning year to year, which may contribute recruitment to the adult stock either in large or in small numbers of individuals. This perhaps is explained by the differences in survival in the early stages of existence of the fish—either in the developing eggs, among the hatched larvæ or in the young fry. The variability in survival affects the abundance of the fish population to a great deal as a poor, fair or abundant brood may be the result of the spawning of any one year. When the young ones from a successful spawning reach an age sufficient to enter the fishery, there is a definite rise in the level of abundance and on the contrary the failure of spawning in one or more years leads to a drop in the abundance level.

The factors which affect the successful spawning and the rate of survival of the young fish are:—

(*a*) the presence of large numbers of spawning individuals in the spawning grounds,

(*b*) the favourable hydrological conditions for spawning and for the survival of the larvæ, and

(*c*) the availability of the food for the fry and young sardines.

In dealing with the occurrence of the mature Oil Sardines along the Coast of Malabar and South Kanara, one should consider the seasonal appearance of the sardine in the areas and from where they migrate.

Details of the first date of appearance and the last date of appearance of the Oil Sardine in the fish curing yards on the West Coast arranged in order from north to south for the fishing season, June to May, for the years 1936–37, 1937–38, 1938–39, 1939–40 and 1940–41 are given in Table IV.

A reference to Table IV indicates in general that the Oil Sardines appear first in the region of Calicut and then appear gradually in succession towards the north and begins disappearing in the north first and then near about Calicut and then in the south. There is a slight mixing up in the period of appearance in the regions of Calicut and the south, the causes for which are to be found in the effect of the monsoon on the mud banks which become turbid and constantly disturbed. The movements need not generally be from south to north but also from off-shore to in-shore depending upon the temperature conditions and the disturbance caused by the effect of monsoon.

The shoreward migration of Oil sardine and the surface temperature of sea water

During the monsoon the fishing is restricted due to the adverse weather conditions. The catches averaging over five hundred maunds of Oil Sardines

in the jurisdiction of a fish curing yard in the months from August of one year to March of the following year during the years 1936–43 are considered and the landings are indicated against the fish curing yard in the maps for each of those months by certain symbols,—*vide* Appendix III. Against Calicut the average temperature for the month during the years 1936–37 to 1942–43 is furnished. The map shows that the fish was caught in quantities more than 1,000 maunds at Calicut in August, September, October, November, December, January and February when the temperature ranged between 26·15° C. to 28·68° C., but they were not taken in March when the temperature rose above 29·0° C.

In the month of August, Oil Sardines were caught in Calicut and to a little extent to the north, in September, they were taken as far as Kumbla in the North and Ponnani in the south; in October, November and December they were found all along the Coast. In January they were taken all along Malabar coast and only in Malpe in the North. In February Oil Sardines appeared in regions south of Calicut and in March they were found in the extreme south. A Table showing monthly average surface temperature of sea water, monthly average specific gravity of sea water and monthly average landings of Oil Sardines at Calicut for the period 1934–35 to 1942–43 is given below:—

TABLE V

Month	Average landings of fish at Calicut in maunds	Average surf. temperature of sea water in °C.	Average Sp. gravity of sea water
July ..	37½	26·1	1·0190
August ..	1610	26·22	1·0209
September ..	2,838	26·70	1·0223
October ..	5,439	28·28	1·0222
November ..	5,491	28·55	1·0222
December ..	9,157	28·03	1 0227
January ..	6,580	28·02	1·0221
February ..	765	28·38	1·0229
March ..	..	29·82	1·0236
April ..	30	30·22	1·0231
May ..	620	29·96	1·0237
June ..	400	27·42	1·0218

Table V shows that the landings of fish from September to February are very good, when the surface temperature had been below 29·00° C. and specific gravity had been less than 1·023. From February to May the catches were negligible. The reason for smaller catches in June and July even though the temperature is low could be attributed to the stormy condition of the sea when fishing could not be conducted except on a few calm

TABLE VI

The monthly average temperature of the sea water at West Hill (Calicut)

Year	Temperature of Sea water in Centigrade											
	July	August	September	October	November	December	January	February	March	April	May	June
1932—33 ..	26·59	28·39	27·81	28·42	27·77	30·14	28·77	30·22	31·74	31·78	31·17	27·0
1933—34 ..	23·59	25·83	26·37	27·11	29·05	28·9	28·61	29·35	29·69	29·69	30·47	26·17
1934—35 ..	25·14	26·53	26·89	28·33	28·11	28·61	28·89	29·58	29·45	29·45	31·28	29·39
1935—36 ..	26·11	26·39	27·5	27·09	28·05	27·22	26·9	27·22	28·51	30·83	30·27	28·89
1936—37 ..	26·38	26·67	27·78	28·33	28·05	27·5	30·0	..	30·83	30·27	30·27	27·61
1937—38 ..	26·94	27·22	27·22	28·61	27·78	27·67	27·22	28·00	30·55	30·00	30·50	26·11
1938—39 ..	25·56	26·38	26·67	28·05	28·05	28·05	27·35	28·02	28·7	29·25	29·25	26·75
1939—40 ..	26·25	26·25	25·86	28·05	28·5	27·7	27·9	28·2	30·45	30·5	30·1	28·15
1940—41 ..	25·75	24·85	26·3	29·25	29·65	28·9	28·45	28·65	30·25	31·2	29·55	27·3
1941—42 ..	26·55	26·32	26·75	28·25	29·9	28·8	27·9	29·1	30·25	30·6	29·55	27·45
1942—43 ..	26·2	25·35	28·35	28·1	28·8	28·8	27·6	28·05	29·4	29·9	28·9	25·1
Average ..	25·91	26·37	26·67	28·18	28·52	28·29	28·14	28·66	29·98	30·31	30·12	27·27

days and in August the catches started increasing in view of the better weather conditions breaking in.

The relationship of the appearance of the Oil Sardines at Calicut with the surface temperature of the sea water in the locality is evident and a similar relationship can be indicated for the west coast of Madras Presidency. The average temperature records for the twelve months of each of the eleven year period between 1932–33 and 1942–43 are given in Table VI.

It shows that the temperature is low in July with a eleven year average of 25·91° C. and gradually increases to 26·37° C., 26·67° C., 28·18° C. and 28·52° C. in August, September, October and November respectively; there is a slight fall to 28·29° C. and 28·14° C. in December and January and again there is a rise to 28·66° C., 29·98° C. and 30·31° C. in February, March and April respectively. In May it tends to fall down a little and in June it falls to 27·27° C. The months of March, April and May register the maximum temperature of the sea water at Calicut when it is above 29·0° C. and when there were practically no landings of Oil Sardines of any importance in the period under study (1935–36 to 1942–43). The variations in the temperature in the different months of the year observed at Calicut resemble very much those recorded by Koninkulijk Nederlandsch Meteorologish Institute (1927–30) and Sewell (1925, p. 282). The differences in the temperature are due to the records of the temperature in different stations and the trend of rise and fall in the different months are similar.

The records of average surface temperature of sea water in ° Centigrade for the months from September to May in the different latitudes along the West Coast of Madras Presidency according to the Nederlandsch Meteorologish Institute (1927–30) are given below:—

TABLE VII

Areas as per latitude	Geographical range	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May
8° N. to 10° N.	Cape Comorin to Cochin	26·0	27·3	28·0	27·5	27·5	27·7	28·8	29·5	28·4
10° N. to 12° N.	Cochin to Palayangadi	26·5 26·7	.. 27·8	.. 28·1	27·4 27·6	.. 27·6	.. 27·8	.. 28·8	.. 29·9	.. 28·5
12° N. to 14° N.	Palayangadi to Northern boundary of S. Kanara	26·5	28·1	27·9	27·5	27·7	28·0	28·3	29·1	29·4

The above Table shows that there is uniform increase in the surface temperature of the sea water on the West Coast of India from 8° N. to

14° N., *i.e.*, from Cape Comorin to the northernmost part of South Kanara, during the months of September and October and January, February and May and it is fairly uniform throughout in November and December. In March the temperature is the same from 8° N. to 12° N. and falls a little in the region between 12° N. to 14° N. and in April the temperature is above 29·0° C. This gradual increase in temperature from southern area to the northern area explains the appearance of Oil Sardines from the region of Calicut and south early in the season and a gradual succession of appearance in the northern regions in the following months and the final disappearance in the same manner retrogressively from north to south. It is not possible to correlate the data on the appearance of the sardine in the months of June and July, because the variations in the catches of Oil Sardines are due to the difficulty of fishing caused by frequent squalls and stormy weather in those months, even though it can be argued and anticipated that the fall in temperature would attract the fish.

(*b*) *Effect of hydrological factors on the spawning and survival of larvæ*

The second factor is the favourable hydrological condition for spawning and survival of the young. The spawning season of the Oil Sardines extends from June to October. One of the spawning grounds of the Oil Sardine is on the coasts off Quilandy (Devanesen, 1943). Among the various environmental factors which might be responsible for the fluctuations of the fishery by affecting the early larval stages of the fish, when the mortality is considered to be high and variable, surface temperature and specific gravity of sea water are considered here. The landings of fish under the jurisdiction of the fish curing yards from Quilandy to Calicut are taken for this study, and also the records of hydrological data as maintained at West Hill to represent the conditions of this region.

The year class which had contributed to the fishery of the following years, the season of capture and the quantity of oil sardine in maunds are presented in the following Table:—

TABLE VIII

Year class	Season of capture	Quantity of Oil Sardine in maunds landed at Calicut–Quilandy
1934	1936—37	1,36,405
1934 & 35	1937—38	91,037
1935 & 36	1938—39	6,120
1937 & 38	1939—40	14,528
1938 & 40	1940—41	1,41,229
1938	1941—42	17,976
1941	1942—43	3,063

The annual average surface temperature and average specific gravity of the sea water at Calicut for the period 1934 to 1942 are given below:–

TABLE IX

Year	Average surface temp. of sea water in °C.	Average specific gravity
1934	26·61	1·0214
1935	27·30	1·0209
1936	27·61	1·0216
1937	27·52	1·0220
1938	26·55	1·0208
1939	26·72	1·0214
1940	26·86	1·0213
1941	27·03	1·0211
1942	27·09	1·0219

The 1934 year class of Oil Sardines, which contributed the fishery of 1936–37, yielded 1,36,405 maunds of Oil Sardines, when the temperature was 26·61° C. The 1935 and 1936 year classes contributed very little to the fishery as also the year classes of 1937, 1938 and 1941 but 1939 and 1940 year classes accounted for the fishery of 1940–41 which amounted to 1,41,229 maunds. The average surface temperatures of sea water at West Hill in the years 1935, 1936 and 1937 are 27·30° C., 27·61° C. and 27·52° C. respectively and in the years 1939 and 1940 the temperatures are 26·72° C. and 26·86° C. It is evident that the spawning survival has been satisfactory in the years 1934, 1939 and 1940 when the range of temperature of sea water has been between 26·61 and 26·86° C. During these years, the range of specific gravity of the sea water at West Hill has been between 1·0213 and 1·0214.

Earlier it was indicated that the dominance of a group in 1937–38 was also due to a fairly good survival of those spawned late in 1935, besides the contribution of 1934 year class and it could have been possible, as the surface temperature in June 1935 was 29·39° C. whereas in July and August it fell down to 26·11° C. and 26·39° C. Further investigations are necessary to find out the effect of the various other factors on the spawning and survival of the young.

(*c*) *Availability of food for the young sardines*

Besides the above two factors, there is a third important factor, *viz.*, availablity of food for the larvæ and fry, which can influence the survival of the young fish. It was reported that " the food of the young Oil Sardine does not materially differ from that of the adult sardines " (Administration

Report of Madras Fisheries Department for the year 1939–40, p. 7). Devanesen (1943) states that the normal food of the Oil Sardine consists of protophytes and dinoflagellates, comprising of *Pleurosigma* spp., *Biddulphia* spp., *Coscinodiscus* spp., *Fragillaria* spp., *Peridinium* spp., and *Ceratium* spp. Hornell and Ramaswamy Nayudu (1924, p. 168) indicate that ' the curve of growth follows that of the abundance of the diatoms, infusorian and dinoflagellate constituents of the plankton.' These records and the investigations of the food of Oil Sardines conducted at West Hill subsequently show that the young sardines feed mainly on *Biddulphia* spp., *Chætoceras* spp., *Coscinodiscus* spp., *Fragillaria* spp., *Dinoflagellates* and *Copepods*. The appearance of these organisms during the period from 1934 to 1943, as recorded in the annual plankton records maintained in the West Hill Biological Station was referred to and they revealed that the above mentioned organisms occurred in abundance in the years 1934. 1935, late in 1938, 1939, 1940 and 1941. In 1936, 1937 and early in 1938 and 1942, they were not in abundance. The favourable conditions of food for the young sardines in 1934, 1939 and 1940 had contributed partially to the successful survival. In 1935, 1938 and 1941 even though the food factor had been favourable, the other hydrographical characters do not seem to have been conducive for a successful survival.

6. Discussion

Hornell and Ramaswamy Nayudu (1924) have been able to trace the growth of the Oil sardine from the earliest stage met with on the Malabar Coast until sexual maturity was obtained and to read the age of the sardines from the scales? They had indicated that the sardines measured 10·2 cm. when they were 6½ months old, 15·0 cm. when they were 18 months old and 16·0 cm. when they were 30 months old and concluded that " this may be taken as the normal limit of the Oil Sardine, for the number of those met with of the size towards the end of 1922 were few and far between ". Devanesen (1943) had stated that " more than two rings are found in the scale of *Sardinella longiceps*. The primordial rings and vestigeal rings have been discovered for the first time in the scales of the Oil Sardine." He had indicated that the Oil Sardines 6·5 cm. long belonged to two year class, Sardines 8·7 cm. long to six year old class and that it was likely that the Sardines measuring 21·0 cm. belonged to 14 year old group. From the present study of the length frequencies and size and age groups of the Oil Sardines of the Malabar coast during the period 1936–37 to 1942–43, it appears that the life of the Oil Sardine is between 3 and 4 years, the average length of the Oil Sardine being 10·0, 14·5, 18·3 and 20·5 cm. in their 1st, 2nd, 3rd and 4th years respectively. It is likely that Devanesen had counted false

rings as a complete ring and thus estimated the age of the sardine. Further the method adopted by him for mounting the scales may have given rise to false interpretations and it is indicated that the method employed by Walford and Mosher (1943) for the study of the age of Californian sardine may be more successful. Nair (1949) working on the otoliths of the Oil Sardines on the lines followed in California is inclined to believe that 'the average life of the fish is about three years'. The age of the sardine when the first ring appears has to be determined and this knowledge will give us more definitely the age of the fish. The fact that the difference in length in specimens having two and three growth rings respectively is 8 mm., and the difference in length in specimens having one and two growth rings respectively is 67 mm., has induced Nair (1949) to investigate at Calicut the otoliths of Oil Sardines of different sizes and the sizes at different ages and to determine the age at which the first ring makes its appearance. These investigations should throw more light on the determination of age of the Oil Sardines and the results will be awaited with considerable interest.

Devanesen (1943) points out that "in the investigations of the migrations of the Oil Sardine, it is necessary to ascertain if there are local races. If so, then the theory of migration cannot be tenable". It is necessary to know whether the Oil Sardines occurring on the West Coast comprise several discrete populations, each limited to certain regions or whether these intermingle. In 1931 the question of the probable existence of local races of *Sardinella longiceps* Cuv. and Val., was raised by Sunder Raj and Devanesen and the latter made efforts to obtain samples from different centres on the coasts of the Arabian Sea. In March 1932, sixty-one specimens were obtained from Karwar; in December 1935 one hundred and six specimens were received from Karachi; in September 1935 twenty specimens from Muscat and in February 1937 one hundred and six specimens from Aden were received. Observations made by Devanesen and Chidambaram (1942) on the various linear measurements and the number of fin rays in dorsal, pectoral and anal fins in the different samples indicated the possibility of the existence of more than one race; they also pointed out the need for examining more specimens to study the vertebral counts and scales.

According to Heincke (1898) the term "race" means a group of individuals living under the same external conditions, together propagating their kind and having a more or less close relationship to each other. Working on this basis he had examined the variations of a large number of factors including the number of vertebræ, keeled scales and number of fin rays. Ford (1928) presented the hypothesis that the causes of the variations in the

number of vertebræ are genotypic or phenotypic, but doubted the adequacy of characters such as the numbers of vertebræ, keeled scales and fin rays to establish the recognition of possible races in herrings. He thought that a more definite knowledge than we have of the parts played by heredity and environment on such 'racial characters' would be necessary. Hubbs (1934) has pointed out that it has been held that fish from warmer waters have a smaller average number of vertebræ than fish of the same species from colder waters. In the light of the interpretation of Heincke referred to above, even though the variations are caused by environmental factors, they can be treated as different populations or 'races'. Pending further work on the 'race' problems and with the existing knowledge, the Oil Sardines of the West Coast of the Madras Presidency are treated for the purpose of the present paper as constituting one population.

On the assumption that the West Coast Sardines belong to one 'race', it is to be expected that the migration will not be a long range one but will be restricted to limited areas. The fluctuations in the fishery may be dependent upon the migration also; for instance if they do not migrate towards regions nearer the shore within the fishing limits of the fisherimen due to certain factors, then the fishery will be poor, even though they may be abundant in the areas beyond. The factors influencing the migration are mainly spawning and feeding and they are controlled also by certain hydrographical factors. Besides, the hydrographical conditions and availability of food for the young affect the spawning and survival of the young. Hornell (1910), reporting on 'the results of a Fishery Cruise along the Malabar Coast and to Laccadive Islands in 1908', said that the in-shore and off-shore movements of the sardine in the different months had synchronized with the prevailing wind conditions. It was also suggested by him that surface temperature and specific gravity of sea water and food of sardines are factors which are likely to influence the migration of the adult and young sardines.

Whether the Oil Sardines striking the coast first in the south move up northwards gradually or whether the fish occurring in the off-shore regions beyond the limits of fishing operations of the local fishermen strike the coast straight from the off-shore to in-shore regions are yet to be investigated in detail. Table X details surface temperatures of sea water in the Laccadive Sea (Sewell, 1929), in the Arabian Sea (Deutsche seewarter Indischer ozean, 1891), in the coastal regions of the Arabian Sea (the Nederlandsche Meteorologish Instituut, 1927), at Trivandrum (Broun, 1906) and at West Hill, Calicut, by the Madras Fisheries.

TABLE X

	Laccadive Sea (Sewell) 1929	Arabian Sea (from Deutsche seewarter Indischer ozean) 1891	Koninkulijk Nederlandsch Meteorologisch Instituut 1927-30	Trivandrum (Broun) 1906	Calicut (average of 11 years) 1932-33 to 1942-43
January ..	27·28° C.		27·4° C.—27·7° C.	27·3° C.	28·14° C.
February ..	27·73° C.	Between 27 °C.—28° C.	27·6° C.—28·0° C.	27·4° C.	28·66° C.
March ..	28·89° C.		28·5° C.—28·8° C.	29·3° C.	29·98° C.
April ..	29·19° C.		29·0' C.—29·9° C.	29·4° C.	30·31° C.
May ..	28·74° C.	Between 28 °C.—29° C. 29 °C.—30° C. (north of Calicut)	28·5° C.—29·1° C.	29·0° C.	30·12° C.
June ..				27·5° C.	27·27° C.
July ..				26·7° C.	25·91° C.
August ..		Between 26 °C.—27° C.		27·0° C.	26·37° C,
September ..			26·0° C.—26·7° C.	27·3° C.	26·67° C.
October ..	26·79° C.		27·5° C.—28·1° C.	27·24° C.	28·18° C.
November ..	28·17° C.	Berween 27 °C.—28° C.	27·9° C.—28·1° C.	27·80° C.	28·52° C.
December ..	28·00° C.		27·4° C.—27·6' C.	27·6° C.	28·29° C.

This Table indicates that the Oil Sardines appear first in the southern region because of lower temperatures than in the north, and that they are likely to move from off-shore to in-shore regions and disappear in the same manner, in view of the higher temperature of the in-shore waters. Further observations in different seasons on Oil Sardine shoals in regions beyond the present fishing limits will lead to a better understanding of the fluctuations of the fishery. If it is proved that the sardines move to the off-shore regions, only because of the higher temperature of in-shore regions, it will be worth while fishing in the off-shore regions.

Sunder Raj (1936), reviewing the work on Oil Sardines in the Administration Report of the Madras Fisheries Department for the year 1934–35, reported that " during the years 1931–35, the 1933–34 season was the most abundant for sardine. The fish reappeared after years of scarcity in large numbers from September to March " and " that the great fishery of 1933–34 was evidently the result of a very favourable spawning season in the beginning of that year and the lean fisheries of 1934–35 were the result of poor spawning in the subsequent years ". The correlation between the unusual hydrological factors in the Arabian Sea in 1933–34 and the abundance of Oil Sardine in that year appears to be significant. James Paten (1936), referring to the charts relating to the temperature data in the Arabian sea in the region of 10° N. latitude (north of Cochin), stated that ' in October, 1933 and January, February and March 1934 sea temperatures in this region tended to be slighty below normal..... The large difference in temperature normally obtaining in February between the colder waters at the head of the Arabian Sea and those at about 10° N. latitude to which the reversal of the current, while the north-east monsoon is still blowing is attributed (Barlow, 1935) was apparently much reduced in 1934 ". Howat (1945) working on the variations in the composition of the sea in West African waters has indicated the probable significance of the influx of the cold water along the coast on the abundance of the catches of the sardines, *Sardinella aurita* and *S. cameronensis* in the area. Walford (1946) has correlated the strength of a year class of the Pacific Sardine, *Sardinops cærulea* with the average daily summer surface salinity basing it on the fact that the salinity reflects the intensity of upwelling which brings up the nutrient materials for plankton, and he has suggested that at the period when the food supply is essential, the young sardine goes through a critical phase of its life history. The specific gravity has been taken into consideration in this paper and the effect of the specific gravity and the availability of food for the adult and the young sardines on the abundance and movements of the Oil Sardines on the West Coast show a striking relationship indicating to a certain extent, the causes for the fluctuations in the fishery.

7. Summary

(1) The studies on the length frequencies, size groups and age groups of the Oil Sardines of Malabar Coast for the period 1936–37 to 1942–43 have been made and the year classes which contributed to the Oil Sardine fishery in the different years are indicated.

(2) The movements of the Oil Sardines and the spawning and survival of the young Oil Sardine on the Malabar Coast seem to indicate a correlation with the surface temperature and specific gravity of sea water and the availability of food. The effect of the surface temperature on the movements of adult sardines month by month and year by year has been indicated.

(3) The variations in the surface temperature in the in-shore regions and off-shore regions and on the coasts are discussed in relation to the appearance of Oil Sardine. It is suggested that further investigations on the age of the Oil Sardine and the occurrence of the fish in the off-shore regions, beyond the present fishing limits, in different months may further aid in appraising the causes for the fluctuations of the Oil Sardine fishery.

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APPENDIX I

Month	Groups in cms.																			Total examined
	3 cms.	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
										1936—37											
November ..	..	..	..	..	..	..	..	..	39	103	52	2	..	4	..	..	..	..	..	..	200
December ..	..	..	..	..	1	18	48	31	17	75	54	6	3	29	18	6	10	16	2	1	335
January ..	..	..	..	..	..	..	..	..	11	90	48	1	..	..	..	..	..	..	..	..	150
June ..	..	..	..	..	..	..	..	..	..	..	..	..	6	71	66	40	5	8	3	..	199
Total ..	..	..	..	..	1	18	48	31	67	268	154	9	9	104	84	46	15	24	5	1	884
Percentage ..	..	..	..	..	·1	2·0	5·5	3·5	7·6	30·3	17·5	1·0	1·0	11·6	9·5	5·2	1·7	2·7	·6	·1	
										1937—38											
July ..	..	..	..	..	..	..	..	..	..	..	..	..	4	92	125	66	9	10	..	..	306
August ..	..	..	..	..	..	..	..	1	..	18	29	2	..	52	109	79	21	8	..	..	319
September ..	..	..	..	..	..	..	63	37	27	86	69	10	4	21	20	10	1	..	..	..	349
October ..	..	..	..	..	..	..	..	..	1	28	148	54	6	12	14	21	6	4	..	..	294
November ..	..	..	..	..	..	..	..	5	48	40	50	142	62	8	54	54	8	6	..	..	477
December ..	..	..	..	..	..	..	..	..	1	22	15	95	104	31	128	43	11	..	..	..	450
Total ..	..	..	..	..	..	..	63	43	77	194	311	303	180	216	450	273	56	28	..	..	2198
Percentage ..	..	..	..	..	..	.	2·9	2·0	3·5	8·8	14·5	14·0	8·1	9·8	20·3	12·4	2·4	1·3	..	..	..

Appendix I—(*Contd.*)

Month	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total examined
										1938—39											
August ..	..	..	..	..	..	..	..	..	20	69	1	..	..	1	9	88	47	3	..	..	238
September ..	..	..	..	..	..	18	163	18	..	1	..	..	..	..	..	20	27	4	..	..	251
November ..	..	..	..	..	..	..	..	..	..	..	3	29	17	10	5	..	2	..	..	..	66
December ..	..	..	..	..	..	..	..	..	..	..	..	16	28	39	14	..	2	1	..	..	100
January ..	..	..	..	..	..	..	..	..	..	..	1	6	21	18	18	2	8	1	..	..	75
February ..	..	..	..	..	..	..	..	..	..	..	..	..	3	10	14	2	2	3	..	..	34
Total ..	..	..	..	..	..	18	163	18	20	70	5	51	69	78	60	112	88	12	..	..	764
Percentage ..	..	..	..	..	..	2·3	21·3	2·3	2·6	9·2	0·7	6·7	9·0	10·2	7·9	14·7	11·5	1·6	..	..	..
										1939—40											
July ..	..	..	..	..	..	..	34	9	24	45	1	..	..	..	..	..	..	..	..	..	113
August ..	..	..	..	..	..	..	34	8	16	42	10	40	3	..	..	..	..	..	..	..	153
September ..	..	..	65	29	126	102	144	109	41	12	..	..	..	..	..	3	37	33	6	..	707
October ..	..	..	..	..	..	..	2	28	38	100	44	2	..	2	..	3	2	2	..	..	223
November ..	..	..	..	..	..	..	..	..	..	6	25	65	3	..	..	3	4	12	1	..	119
December ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10	25	16	20	2	..	73
January ..	..	..	..	..	..	..	..	..	..	..	12	21	8	1	..	..	..	..	..	..	42
February ..	..	..	..	..	..	..	..	..	..	5	36	35	7	1	..	..	..	..	..	..	84
March ..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	2
April ..	..	..	..	..	..	..	..	..	..	..	..	2	22	11	15	11	..	..	..	..	61
May ..	..	..	..	..	..	..	..	..	..	..	..	18	83	24	18	2	1	..	..	..	146
Total ..	..	..	65	29	126	102	214	154	119	210	128	183	127	40	43	45	60	67	9	..	11723
Percentage ..	..	..	3·8	1·7	7·4	5·9	12·4	8·9	6·9	12·2	7·4	11·7	7·4	2·3	2·5	2·6	3·5	3·9	0·5	..	

											1940	41									
July ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	10	..	..	..	12
August ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	20	8	..	..	..	30
September ..	52	64	8	..	..	..	..	..	..	..	..	..	..	..	..	1	9	8	5	1	..	148
October ..	..	..	..	..	..	..	14	37	31	34	26	13	..	..	..	..	..	..	..	..	..	155
November ..	..	..	..	..	..	..	..	13	52	52	122	186	22	1	..	34	60	17	8	..	..	567
December ..	..	..	..	..	..	..	..	1	19	61	115	177	37	14	9	103	97	13	1	..	..	647
January ..	..	..	..	..	..	..	..	23	46	123	219	284	35	..	..	..	..	..	..	..	..	730
February ..	..	..	..	..	..	..	..	..	20	90	103	31	6	..	..	..	..	..	..	..	..	250
Total ..	52	64	8	..	..	..	14	74	168	350	585	691	100	15	12	168	183	35	10	..	..	2539
Percentage ..	2·0	2·5	0·3	..	..	..	0·6	2·9	6·7	13·8	23·1	27·3	3·9	0·5	0·2	6·7	7·3	1·4	0·4	..	..	..
										1941—42											
July ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	6	61	33	15	1	..	116
August ..	..	..	..	..	..	..	..	..	2	1	1	1	..	..	3	32	32	33	5	..	..	110
September ..	..	..	..	..	..	..	3	62	3	..	..	..	..	2	..	23	39	11	2	..	..	145
October ..	..	..	..	..	..	..	..	..	..	..	..	2	40	107	50	4	16	12	5	..	..	236
November ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	8	11	3	..	..	..	25
December ..	..	..	..	..	..	..	..	..	..	..	2	9	46	57	25	6	4	1	..	..	..	150
January ..	..	..	..	..	..	..	..	..	..	..	..	..	5	50	46	8	7	12	4	..	..	132
Total ..	..	..	..	65	29	126	102	214	154	119	210	128	183	127	40	43	45	60	67	9	..	1146
Percentage ..	..	..	..	3·8	1·7	7·4	5·9	12·4	8·9	6·9	12·2	7·4	11·7	7·4	2·3	2·5	2·6	3·5	3·9	0·5	..	11723

APPENDIX I—(*Contd.*)

Month	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total examined
February ..	..	..	..	..	..	..	..	..	..	..	1	3	4	1	..	..	..	..	..	..	9
May ..	..	..	..	..	..	..	..	..	..	..	..	..	6	14	7	..	..	..	..	..	27
June ..	..	..	..	..	..	..	..	..	..	..	..	..	11	54	14	7	..	1	..	..	87
Total ..	..	..	..	..	..	3	62	5	1	3	13	94	237	202	163	149	87	18	..	..	1037
Percentage ..	..	..	..	..	..	0·3	6·0	0·5	0·1	0·3	1·2	9·1	22·8	19·5	15·7	14·4	8·4	1·7	..	..	..
									1942—43												
September ..	..	..	..	..	..	..	..	..	3	8	31	44	..	10	25	9	1	..	..	..	131
October ..	..	..	..	..	..	..	..	..	..	4	16	72	9	..	..	..	..	..	..	..	101
November ..	..	..	..	..	..	..	..	3	5	7	29	7	12	2	12	13	5	1	..	..	96
Total ..	..	..	..	..	..	..	..	3	8	19	76	123	21	12	37	22	6	1	..	..	328
Percentage ..	..	..	..	..	..	..	..	0·9	2·4	5·9	23·3	37·1	6·5	3·7	11·3	6·8	1·8	0·3	..	..	..

APPENDIX I—(*Contd.*)

Average Frequencies and the Deviations from the Average during the Seven Fishing Seasons—1936–37 *to* 1942–43

Year	3 cms.	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22 cms.
1936—37	..	..	..	..	−0·97	0·54	−0·64	−0·16	3·5	19·33	7·59	−11·03	−9·8	2·66	0·37	−4·6	−5·1	−0·31	0·1	0·09
1937—38	..	..	..	..	..	..	−3·24	−1·66	−0·6	− 2·17	4·59	1·97	−2·7	0·66	11·17	2·6	−4·4	−1·71	..	..
1938—39	..	..	..	..	..	0·84	15·16	−1·36	−1·5	− 1·77	−9·21	− 5·33	−1·8	1·06	−1·23	4·9	4·7	−1·41	..	..
1939—40	..	..	3·21	1·46	6·33	4·44	6·26	5·24	2·8	1·23	−2·51	− 1·33	−3·4	−6·84	−6·63	−7·2	−3·3	0·89	..	..
1940—41	1·71	2·14	−0·27	..	..	..	−5·54	−0·76	2·6	2·83	13·19	15·27	−6·9	−8·54	−8·63	−3·1	0·5	−1·61	−0·1	..
1941—42	..	..	..	..	..	..	−5·84	2·34	−3·6	−10·87	−9·61	−10·83	−1·7	13·66	10·37	5·9	7·6	5·39	1·2	..
1942—43	..	..	..	..	..	..	..	..	−3·2	− 8·57	−4·01	11·27	26·3	−2·64	−5·43	1·5	..	−1·21	−0·2	..
Average frequency	0·29	0·36	0·59	0·24	1·07	1·46	6·14	3·66	4·1	10·97	9·91	12·03	10·8	9·14	9·13	9·8	6·8	3·01	0·5	0·014

APPENDIX II

Size grouping of Oil Sardines examined month by month

Name of month	Length less than 10·0 cms.		Length 10·1 to 15·0 cms.			Length 15·1 to 20·0 cms.		Length above 20·0 cms.	
		*I.G.	♀	♂	I. G.	♀	♂	♀	♂
			1936—37						
November ..	..	..	..	..	196	22	22	..	..
December ..	..	93	..	..	156	35	38	12	1
January ..	..	..	33	57	125	..	..	..	..
June ..	..	..	..	..	..	83	113	3	..
			1937—38						
July ..	..	..	..	..	..	195	156	1	1
August ..	..	..	..	..	..	135	133	1	..
September ..	..	97	..	..	195	27	29	1	..
October ..	..	..	..	..	236	27	30	..	..
November ..	..	..	31	27	186	39	43	..	..
December ..	..	..	..	..	175	102	98	..	..
January ..	..	..	..	..	..	18	29	..	2
			1938—39						
August ..	..	..	..	..	90	82	64	..	..
September ..	..	199	..	..	1	46	5	..	..
November ..	..	..	21	27	1	12	4	..	..
December ..	..	..	24	9	3	26	38	..	..
January ..	..	..	10	11	..	26	28	..	..
February ..	..	..	1	2	..	19	8	..	..
			1939—40						
July ..	..	..	..	..	..	7	8	..	..
August ..	..	42	..	..	111	..	..	..	..
September ..	..	561	..	..	37	32	34	11	2
October ..	..	17	2	..	55	3	5	1	..
November ..	..	..	47	42	..	9	7	4	..
December ..	..	..	..	..	..	43	22	5	3
January ..	..	..	16	8	16	1	1	..	..
February ..	..	..	25	28	30	1	..	..	..
March ..	..	..	..	..	..	2	..	..	..
April ..	..	..	10	6	..	29	15	..	..
May ..	..	..	21	38	..	37	38	..	..
			1940—41						
July ..	..	..	..	..	..	7	5	..	..
August ..	..	..	..	..	..	18	12	..	..
September ..	..	124	..	..	..	13	9	2	..
October ..	..	45	14	1	95	..	..	..	..
November ..	..	..	..	..	347	73	41	5	1
December ..	..	..	5	1	404	120	116	1	..
January ..	..	..	..	..	730	..	..	..	..
February ..	..	..	..	..	250	..	..	..	..
			1941—42						
July ..	..	..	..	..	..	59	59	1	..
August ..	..	..	..	..	4	62	36	6	1
September ..	..	..	..	..	..	40	30	6	1
October ..	..	..	8	8	..	45	45	6	4
November ..	..	..	..	..	..	14	9	2	..
December ..	..	..	9	13	..	59	68	..	1
January ..	..	..	..	2	..	40	47	8	2
February ..	..	..	..	..	..	12	15	..	..
June ..	..	..	..	..	..	46	40	1	..
			1942—43						
September ..	..	..	66	33	..	39	28	..	..
October ..	..	..	..	..	95	..	..	..	..
November ..	..	..	17	3	32	28	13	..	..

* I.G. = Indeterminate gonads.

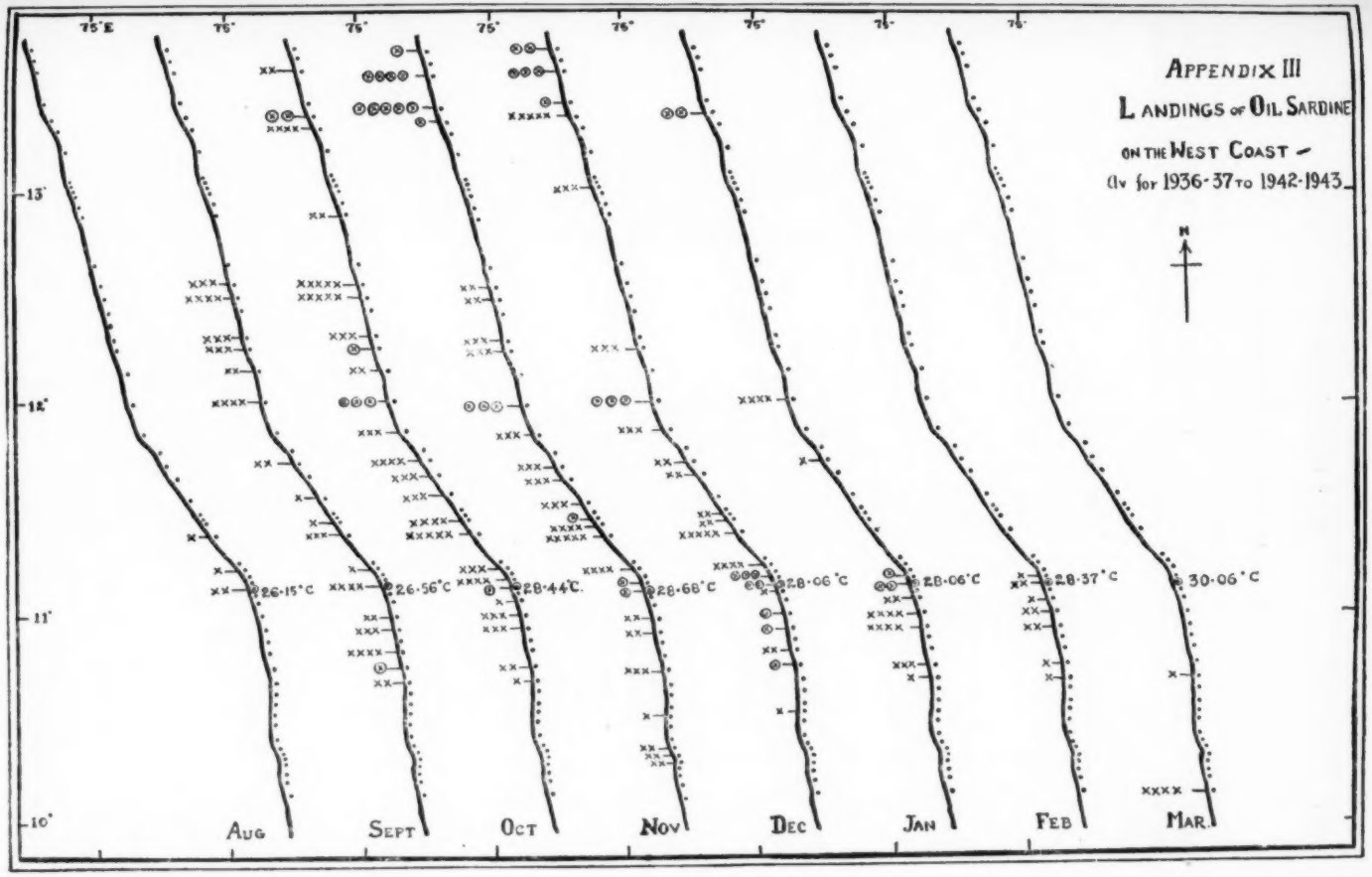
APPENDIX III
LANDINGS OF OIL SARDINE
ON THE WEST COAST —
Qty for 1936-37 TO 1942-1943
N
75°E
75°
13°
12°
11°
10°
26·15°C
26·56°C
28·44°C
28·68°C
28·06°C
28·06°C
28·37°C
30·06°C
AUG
SEPT
OCT
NOV
DEC
JAN
FEB
MAR.

FIG. 4.

Explanaton to Appendix III

Maps of the West Coast of Madras Presidency showing the average landing of Oil Sardines more than 500 maunds in the various fish curing yards for the period 1936–37 to 1942–43 in the different months from August to March:—

(*a*) 1.	Between	500	and	1,000	maunds	..	..	X
2.	,,	1,000	,,	2,000	,,	..	..	XX
3.	,,	2,000	,,	3,000	,,	..	..	XXX
4.	,,	3,000	,,	4,000	,,	..	..	XXXX
5.	,,	4,000	,,	5,000	,,	..	..	XXXXX
6.	,,	5,000	,,	7,500	,,	..	..	(X)
7.	,,	7,500	,,	10,000	,,	..	..	(X)(X)
8.	,,	10,000	,,	15,000	,,	..	..	(X)(X)(X)
9.	,,	15,000	,,	20,000	,,	..	..	(X)(X)(X)(X)
10.	,,	20,000	,,	25,000	,,	..	..	(X)(X)(X)(X)(X)

(*b*) The names of yards indicated by dots from north to south:—

1. Kirimanjeshwar
2. Maravante
3. Gangoli
4. Hungarcutta
5. Malpe
6. Udayawar
7. Moolki
8. Hosabettu
9. Baikampadi
10. Thannirbavi
11. Bockapatnam
12. Mangalore
13. Ullal
14. Manjeshwar
15. Kumbla
16. Adakathbail
17. Kasargod
18. Bekal
19. Hosdrug
20. Thaikadapuram
21. Madai
22. Mattool
23. Azhicode
24. Cannanore
25. Edakkad
26. Tellicherry
27. Thalayi
28. Madakkara
29. Madapalli
30. Badagara
31. Meladi
32. Quilandy
33. Elathur
34. Pudiappa
35. Calicut (north)
36. Beypore (north)
37. Beypore (south)
38. Parapanangadi
39. Tanur
40. Puthenkadapuram
41. Paravanna
42. Koottayi
43. Ponnani
44. Puduponnani
45. Veliangode
46. Palapetty
47. Mannalamkunnu
48. Puthiakadapuram
49. Blangad
50. Kadapuram
51. Vadanapally
52. Nattika
53. Kaipamangalam
54. Karimpuram
55. Kodunbi

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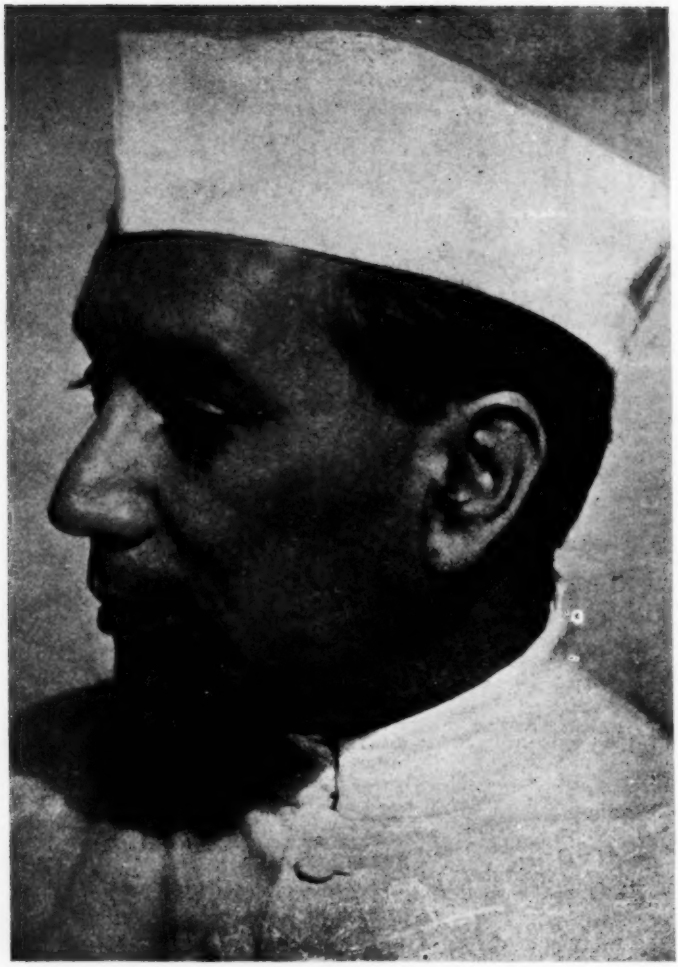

PROFESSOR BIRBAL SAHNI

OBITUARY NOTE

PROFESSOR BIRBAL SAHNI, Sc.D. (Cantab.), F.R.S.
(1891–1949)

THE premature demise of Professor Birbal Sahni on 10th April 1949 at the early age of 58 is a tragedy of the first magnitude not only to the Republic of India, but to the cause of Science all the world over; and the void caused by his death cannot be easily filled. When all the eyes of the scientists of India and of other nations were turned towards the Sahni Institute of Palæobotany in 1949—an institution unique of its kind in the world with a most inspiring Director as its head—the cruel hand of destiny dealt a blow, the consequences of which are hard to imagine.

Professor Birbal Sahni was born on the 14th November 1891, at Bhera, Shahpur District, West Punjab, adjacent to the Salt Range, the stratigraphy and tectonics of which have attracted the attention of eminent geologists of international repute, including Professor Sahni himself, during the later part of his life. Professor Sahni's grandfather, Lala Karamchand Sahni, was a flourishing banker by profession with experimental alchemy as his hobby. Professor Sahni was the second son of Professor Lala Ruchiram Sahni and Srimathi Eswari Devi. Ruchiram Sahni who retired in 1918 as the Professor of Chemistry from the Government College, Lahore, and died only a few years ago, spent his retirement in public service, social reform activities, and in clutural pursuits. His reputation for scholarship and character was high all over India, and he was a familiar figure in political and scientific circles.

Professor Birbal Sahni's first education was under his great father and even as a child he displayed extraordinary interest in nature study including the collection of leaves, shells, rocks and minerals. The father, recognising his child's talents, not only encouraged him in these pursuits, but often took him during his tours to the mighty Himalayas. The lure of the Himalayas attracted Professor Sahni to the end of his life and almost every year he would sojourn from the turmoil, heat and dust of the plains, to the cool and serene heights of Kashmir where some of his most important researches and intellectual pursuits were carried out in an atmosphere of Nature's grandeur and the company of his charming and devoted spouse, Srimathi Savitri Sahni.

After his early education at the Central Model School at Lahore, Professor Sahni joined the Government College as a student of Botany under Professor Kashyap, one of the most inspiring teachers and researchers in India of that generation. This happy early association of Professor Sahni was a landmark in his career. After graduating in Science in 1911

from Punjab University, Birbal Sahni proceeded to the Emmanuel College, Cambridge, for Tripos in Natural Sciences. Besides Botony, he studied Geology for his Tripos, and despite his protestations to the contrary he was a discerning geologist. After obtaining the Tripos, he joined Professor Sir A. C. Seward as a Research Scholar and remained with him throughout the war. Professor Seward was even at that time a leading Palæobotanist with an international reputation. It is no wonder that one so gifted and charming as Birbal Sahni should win the admiration, esteem and life-long friendship of his Professor Sir A. C. Seward. He had a very distinguished record at Cambridge as a Founder-Scholar, Exhibitioner and the winner of the Sudbury Hardyman Prize. He spent some summers at Munich under Professor K. Goebel. He was awarded the Doctorate in Science of the London University in 1919; and the Cambridge University conferred on him the Sc.D. degree in 1929, in recognition of his brilliant researches.

Professor Sahni returned to India in 1919 and held the Chair of Botany at the Universities of Benaras and the Punjab for a year in succession. He continued to hold the Honorary Professorship of Benaras Hindu University to the end of his life. In 1920 he married Savitri Suri, younger daughter of Sri. Sundara Das Suri of the Punjab Educational Department. In 1921 he was appointed Professor of Botany at the University of Lucknow and from 1933 onwards he was also the Dean of the Faculty of Science.

The Botany Department at Lucknow under Professor Birbal Sahni soon became a place of pilgrimage for those seeking higher botanical and palæobotanical education. Students from all parts of India clustered round him, and despite his own preoccupations, he encouraged generously every seeker after knowledge by precept and example. He practically spent his whole life during those years in his laboratory, ably assisted by Mrs. Savitri Sahni. He did not consider any part of the work required for research as too trivial, and was an adept in section-cutting, sketching clay moulding, and photography. Between 1920–26, he spent the summer holidays in the study of the fossil plant collections of the Geological Survey of India at Calcutta. He developed in his students the qualities for which he was himself deservedly famous, *viz.*, initiative, correct observation, meticulous care of details, a critical attitude, and a thoroughness of work. He had an open mind on any problem, and never came to hasty conclusions. It was therefore not easy to shake a position which he generally took after due deliberation and a judicious sifting of facts. He did not try to smother or minimise the opponent's point of view, but always met it squarely and fairly; in spite of the extreme courtesy and politeness in his expressions, the effect of his arguments was often devastating to the cause he opposed.

Professor Sahni's first and foremost love was Palæobotany; but his contributions on the living plants were of no less importance, specially in the field of morphology and evolutionary trends of the Pteridophyta and the Gymnosperms. Some of his important papers on living plants are "Observations on the Evolution of Branching in Filicales" (1917), "Modern Psilotaceæ and Archaic Terrestrial Plants" (1923), "The Ontogeny of Vascular Plants and Theory of Recapitulation" (1925). While at Cambridge, he brought out an Indian edition of "Lowson's Text-book of Botany" in the Cambridge University Series, which to this day is unrivalled for its excellence as an introduction to the study of plants with particular reference to India.

Of his contributions in Palæobotany, it is not easy to do justice in this short note. His first paper of Palæobotany was on "Zygopteridian Leaf", published in *Ann. Bot.*, 1918. Since then in his innumerable papers, he has enriched palæobotanical literature, and given it a status in the deciphering of controverted stratigraphic problems as few had been able to do. While at Cambridge, he commenced his studies in collaboration with Professor Seward on a critical examination of the Gondwana plants. The results of these investigations were published in the *Palæontologica Indica*, new series Vol. VII, 1920. In 1928 and 1931, his exhaustive memoirs on the Indian Fossil Conifers were published in the same Journal. In several contributions in the *Philosophical Transactions of the Royal Society of London*, the results of his investigations on fossil plants from Australia, Western Siberia, and other countries were published. His contributions on Gondwana land Permo-carboniferous, Life-Provinces, Palæogeography, Wegener's Theory of Continental, Drift, the Himalayan Uplift, and the Eastward Opening of the Himalayan Geosyncline, all based on his Palæobotanical researches, are classical in the realm of Geology, and stand on solid foundations. The stratigraphical horizons in India which attracted much of his attention were the Rajmahal Hills of Bihar, the intertrappeans in the Deccan Traps, the saline series of the Salt Range of the Punjab, and the post-Tertiaries of the Kashmir Himalayas. Though most of his work on Palæobotany was carried out in his laboratories, he believed in field studies and the collection of material by himself and his associates for investigation. Thus, Rajmahal Hills in Bihar, Intertrappeans in Central Provinces and the Salt Range and Himalayas were his happy hunting grounds, where he would spend some of his vacations with his band of devoted scholars, forgetful of his health and sometimes, even to the anxiety of his dear and near ones. His close acquaintance with the field relations of the problems in which he was interested as a Palæobotanist, gave him the additional advantage that he had seen all that a field geologist

could see ; and if he differed from the field geologist on the results of his palæobotanical researches, it was after taking due congnisance of the field relations also, though out of modesty, he would not stress on this aspect. This gave him a unique advantage in the controversies on some of the stratigraphic problems, such as the classification of the Gondwanas, the Age of the Deccan Traps, and the Age of the Saline Series. The sheer discovery of several new species of fossil plants and their description and correlation earned for him the foremost place as a Palæobotanist. The application of these studies to the elucidation of controverted problems in stratigraphical geology has made his work doubly valuable. His positive contributions to Indian stratigraphy are that the Deccan Traps are tertiary in age as against the Mesozoic Age advocated by the Geologists of the Geological Survey of India, and that the Saline Series of the Punjab are of Tertiary Age and not Cambrian as believed by some of the Officers of the Geological Survey of India and others. It is perhaps his interest in the Saline Series that drew him closely to the study of microfossils the technique of which he developed in his laboratories with a degree of precision, and care of detail the subject demands. Such researches were also extended in his laboratories to the field of Economic Geology, for purposes of correlation of coal-bearing and oil-bearing strata.

Professor Sahni's publications number over 130, and when it is realised that nothing went out of his hands to the Press which had not something unique or new, and about the accuracy of which, there could be no doubt, it is a record of amazing scientific activity, and achievement. His papers were published in international journals of repute, such as *Proceedings and Philosophical Transactions of the Royal Society of London*, *Annals of Botony*, *Palæontologia Indica*, *Records and Memoirs of the Geological Survey of India*, *New Phytologist*, *Proceedings of International Botanical Congress*, *Nature*, *Journal of the Indian Botanical Society*, *Proceedings of the Royal Asiatic Society of Bengal*, *Proceedings of the National Academy of Sciences*, *Proceedings of the Indian Academy of Sciences* and *Current Science*.

Professor Sahni is the founder of the Indian School of Palæobotany; and with his characteristic intellectual generosity, he induced and encouraged several individuals working in different institutions to take up similar studies for which his spontaneous help and inspirational guidance were ever available. The Bangalore School of Palæobotany including Mr. K. Sripada Rao, Mr. S. R. Narayana Rao and others under the leadership of Professor L. Rama Rao, which made some positive contributions to Indian stratigraphical problems, owed its inspiration to him. The writer of this note records his debt of gratitude to Professor Birbal Sahni for the facilities and

guidance given to him to be initiated into palæobotanical research at Lucknow in 1943, when he went to work with his collections of Intertrappean plant fossils from Hyderabad State. Several of Professor Sahni's students, such as Dr. Shukla at Nagpur, Dr. Jacob and Dr. Mrs. Jacob at Calcutta, Dr. Sadasivan at Madras, Dr. Puri at Dehra Dun, Dr. Ganju at Durham, Dr. Venkatachari at Udaipur and Prof. T. C. N. Singh at Annamalainagar are doing good work in their respective spheres. The School of Palæobotany at Lucknow has a group of brilliant researchers of whom Dr. Sitholey, Dr. A. R. Rao, Dr. Surange, Mr. Lakhanpal, Dr. Hsü, Prof. S. R. Narayana Rao, Mr. Trivedi and Dr. Varma are a few.

Honours came to Professor Sahni unsought and he wore them lightly. He was elected President of the Botanical Section of the Indian Science Congress in 1921, and again at the Jubilee Session in 1938. He was also elected President of the Geology Section of the Indian Science Congress in 1926 and General President of the Indian Science Congress in 1940. He was a Foundation Fellow of the Indian Academy of Sciences, the National Academy of Sciences, the National Institute of Sciences, and the Indian Botanical Society and was a Vice-President of the Indian Academy of Sciences and National Institute of Sciences, and the President for some terms of the National Academy of Sciences and Indian Botanical Society. The Presidential Addresses delivered by him on several occasions at the Science Congress and Academies constitute most important contributions in the realm of Palæobotany, Stratigraphy and Botany. They combined a mature assessment of scientific truths and discoveries with a literary charm and clarity which characterise his writings. He was associated with several Indian Universities and was constantly consulted by Dr. C. R. Reddy, Ex-Vice-Chancellor of the Andhra University, on the organisation of Botanical Education and Research; and he was a Member of the Andhra University Commission, which inspected the Colleges in the Andhra area. He was awarded the Cattamanchi Ramalinga Reddi National Prize, an honour reserved for savants and his was the first award for Natural Sciences, those in Physics and Mathematics who preceded him being Prof. Sir C. V. Raman and Prof. S. Chandrasekhar. He was elected to the Royal Society in 1936 and was the fifth Indian and only Natural Scientist in this country to share this honour. In the international field he was a Fellow of the Geological Society, an Honorary Member of the American Academy of Arts and Sciences, Vice-President of the Botany Section of the International Botanical Congress at Cambridge in 1930 and Amsterdam in 1935. He was elected the Honorary President of the International Botanical Congress to be held in 1950—the greatest honour that could be bestowed on a Botanist. The Royal Aisatic

Society of Bengal awarded him the Barclay Medal for Biological Sciences. He was a keen student of Archæology and made discoveries of far-reaching importance in unearthing ancient mints and moulds for coins. In recognition of this work he was awarded in 1945 the Nelson Wright Medal of the Numismatic Society of India. Only recently Mrs. Savitri Sahni is reported to have presented his valuable archæological collections through Pandit Jawaharlal Nehru to the National Museum at Delhi. The Universities of Patna and Allahabad conferred on him the Honorary D.Sc. degree. He was the Founder President of the Sahni Institute of Palæobotany at Lucknow for the starting and running of which he and Mrs. Savitri Sahni gave a munificent endowment; the work that he did for nearly a decade in focussing the attention of the world on palæobotanical contributions from India by his annual publications of "Palæobotany in India" acquired an honoured place in the international sphere. Professor Sahni was invited by the Andhra University to deliver the Alladi Krishnaswami Aiyar Lectures in 1947 and his demonstrations and addresses on Micro-Palæontology were most popular with all teachers and students of the University, and will be remembered for a long time to come. With the exception of Prof. Sir C. V. Raman, there is hardly an Indian Scientist who could hold his audience, popular or academic, spell-bound by discourses and demonstrations as Professor Sahni. Professor Sahni's lectures were well thought out, his diction and manner of delivery so beautiful and his experiments so carefully planned that they created a profound impression. He gauged his audience and quickly adapted himself to their standard.

It is not easy to pay adequate homage to the human side of Professor Sahni's life. He had an innate charm and pleasing personality that endeared him to all; it was a privilege to come into contact with him. His sincerity was transparent and his patriotism, of the highest type. His relationship with individuals was absolutely free from racial, provincial or communal parochialism. There was something so noble and so genial in his bearing that marked him out distinctly from others. He had a delicate sense of humour and his kindness and intellectual generosity to those who sought his help was unbounded. Professor Sahni was a great unifying force in the scientific world of India. The joint annual deliberations of the Indian and National Academies of Sciences between 1943 and 1946 at Hyderabad, Poona, Udaipur and Allahabad, were the collaborative result of Professor Birbal Sahni and Prof. Sir C. V. Raman, and some of the outstanding contributions to scientific knowledge were made in these meetings, by Indian and foreign scientists.

This memoir will be incomplete without a reference to Mrs. Savitri Sahni, the devoted and affectionate partner in life of Professor Birbal Sahni,

ho, in keeping with the traditions of the great Sadhvi of whom India is proud, and whose name she bears, was the constant friend, companion and helpmate of her husband, and inspired him in no small measure to his personality and achievements. No one who had the privilege of experiencing the hospitality of the Sahnis can forget the charm, simplicity, culture and warmth of heart that emanated from the great scientist and his noble wife. To her falls the burden of carrying out the long-cherished dream of her distinguished husband, namely, the Sahni Palæobotanical Institute at Lucknow.

Professor Sahni visited Europe several times, and he went to America in 1948, for a first-hand study of the latest developments in his subject. With the late Professor Ramanujam, Professor Sir C. V. Raman and Professor Chandrasekar, Professor Sahni shares the honour and glory of having given his Motherland an honoured place in the world of Science. Sahni's mortal remains are no more but he is one of the eternals, whose work will live as long as Science lives and whose memory will always be cherished with deep love and admiration by his innumerable friends and students.

C. MAHADEVAN.

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ON A NEW CALIGID PARASITE FROM THE INDIAN HAMMERHEAD SHARK

By T. S. Satyanarayana Rao, M.Sc.

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Locality and Host

Three females of this parasite were taken from the body of a Hammerhead shark in December 1947, landed in Lawson's Bay, Waltair, South India. The parasites were found attached to the region of the body behind the pectoral fins. In the living condition they were of an yellowish brown colour. The egg sacs were slightly blackish white in colour.

Description of the Female (Figs. A & B)

Measurements

Total length of the body (including the egg sacs) ..	13·00 mm.
Length of the cephalothorax	7·5 ,,
Greatest breadth of the cephalothorax	8·00 ,,
Length of the genital segment	6·5 ,,

Carapace broadly circular, slightly broader than long and of about the same length as the rest of the body; frontal plates not well defined and the demarcation between these and the carapace is very indistinct. Posterior sinuses are very large, conspicuous and extend inward up to $\frac{1}{3}$ of the length of the carapace from the posterior end. The median lobe of the carapace is about one-third of the body width and does not bear any spines. It broadens out posteriorly and reaches the lateral lobes. There are two small oval flaps which originate from the inward margin of the carapace, at the junction of the median lobe with the lateral lobes.

The grooves bordering the areas of the carapace are complexly arranged and constitute the most important single factor in producing the beautiful ornamented pattern of the dorsal surface of the carapace. There are two sets of grooves, the lateral and the transverse; the former in graceful curves. The anterior pair of these curves form a semi-circle with the help of a groove which passes in front of the eye. Running almost across the carapace are the transverse grooves dividing the entire surface into three regions of which the last is of about equal in extent to the front two together. A pair of oblique grooves radiate from either side of the eye towards the frontal

plates. The ocular area is surrounded by indistinct lines (not shown in the figure) forming an oval space. Certain other minor grooves mark out definite quardrangular areas along the dorsal midline of the carapace. The carapace is smooth and there are no spines or setæ of any kind. Bordering the lateral margins of the carapace is a continuous and an almost transparent membranous fringe.

The free thoracic segment is diamond-shaped and is broader than long. It gives dorsally two plates which have a speckled appearance and they extend backwards over the genital segment. Their base is narrow and the free end is broad and cordate. They are leaf like, semitransparent, smooth and devoid of spines.

The genital segment is narrow anteriorly where it joins the free thoracic segment, widens behind, rather abruptly to more than half the width of the carapace. It is prolonged backwards in the form of two sturdy pointed projections, heavily spinose and laterally into broad wing-like expansions, very characteristic of this form. These wings or alata are provided with a few strong spines at the posterior lateral corners. Along the posterior border of the wings are other spines which become gradually smaller.

The abdomen is small, single segmented, with two prominent lateral lobes, best seen from the dorsal side. The anus occupies the hind end of the abdomen. The anal laminæ are foliaceous, bearing two or three small setæ at their tips.

Appendages

1*st Antennæ*.—They are concealed; in each antenna the distal segment is very short, plumpy and bears a few setæ.

2*nd Antennæ* (Fig. 1).—These are large and the basal segments are quite stout; each is two segmented and there are no accessory setæ or spines over it; the terminal claw is slightly curved and turns deep brown in alcohol.

1*st Maxillæ* (Fig. 2).—Each is non-segmented and is triangular in shape; the base is very broad and the pointed angle is very nearly 90°. A circular structure is noticed in the centre of the triangular area.

2*nd Maxillæ* (Fig. 3).—These are conical in shape; but do not end in a sharp point; spines and setæ are absent, their surfaces being smooth.

Mouth is in the form of a conical tube which is very much pointed. The mandibles are styliform and simple. There is no furca.

1*st Maxillipedes* (Fig. 4).—Each is two segmented; the segments being equal in length. The inner claw is half as long as the outer.

2nd Maxillipedes (Fig. 5).—Each is two segmented; there is a hollow depression in the beginning of the first basal segment which gives it a three-segmented appearance. The distal segment ends in a very strong claw which is bifid.

Swimming legs (Figs. 6, 7, 8).—It is noteworthy that this form has only three pairs of swimming legs. The first pair of swimming legs are weak and slender. In each leg the exopod consists of three segments (the distal two are fused) of which the distal bears an outer spine and three inner plumose setæ, which extend inwards upto the tip of the endopod. The endopod is two segmented, the condition being indicated by a constriction behind the rounded tip. The distal segment also bears three plumose setæ directed inwards. The second pair of swimming legs have an exopod of two segments and an endopod of three. All of them bear setæ and spines.

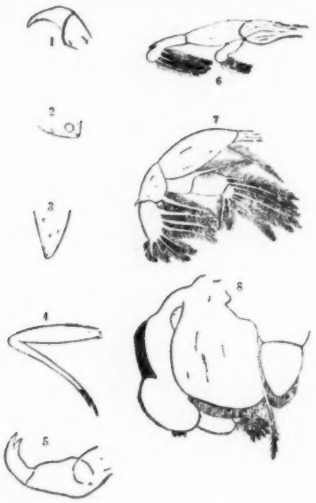


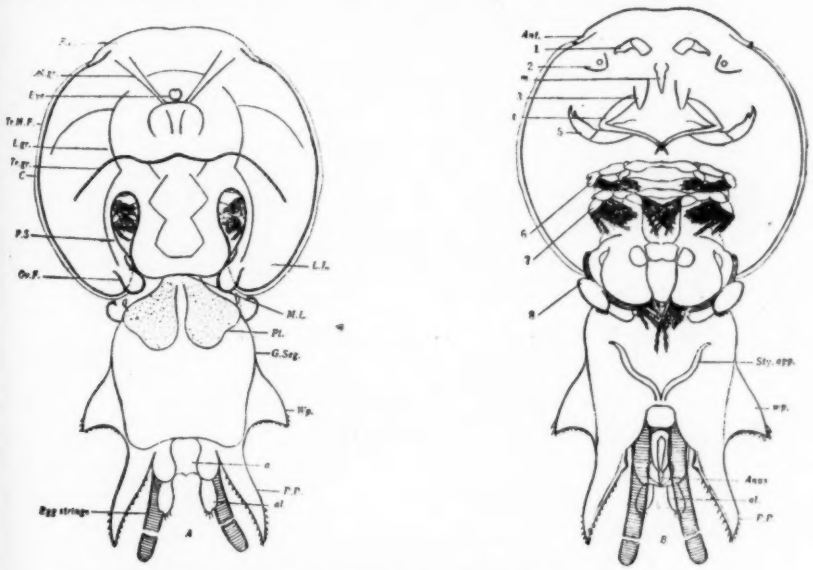
Fig. 1. 2nd Antennæ
Fig. 2. 1st Maxillæ.
Fig. 3. 2nd Maxillæ.
Fig. 4. 1st Maxillipede.
Fig. 5. 2nd Maxillipede.
Figs. 6, 7, 8. 1st, 2nd and 3rd pair of swimming legs.

The third pair of swimming legs are very similar to those of *Gloiopotes*, but the bifid spines characteristic of this genus are absent. The inner rami of third legs are very close with a conical plate in-between. A membranous fringe, delicately striated, is seen along the outer margin of the rami and the

posterior margin of the central plate. The spines and the plumose setæ on the rami have the following arrangements:—

1st Exopod	.. 0:1:3;	Endopod	.. 0:3;
2nd Exopod	.. 2:7;	Endopod	.. 1:2:6;
3rd Exopod	.. 0:4;	Endopod	.. 1:5.

The egg strings are moderately long and uniseriate. They do not, however, project far beyond the tips of the pointed projections (P.P.) of the genital segment. An important feature of the parasite is the presence of two sinuate, styliform appendages arising close together from the midventral line of the genital segment and directed forwards. In the absence of the developmental stages of the parasite the homology of these appendages is not clear. Possibly they are the modified 5th pair of appendages.



Figs. A and B are the dorsal and ventral views of the parasite.

a.	Abdomen	*M.L.*	Median lobe
al.	Anal lamina	*Obl. Gr.*	Oblique groove
Ant.	1st antenna	*Ov. F.*	Oval flaps
C.	Carapace	*Pl.*	Plate
F.P.	Frontal plate	*P.P.*	Pointed projections
G. Seg.	Genital Segment	*P.S.*	Posterior sinuses
L. Gr.	Lateral groove	*Tr. M. F.*	Transparent membranous fringe
L.L.	Lateral lobe	*Sty. app.*	Styliform appendages
m.	mouth	*W.P.*	Wing-like expansion

Systematic Position

The broad carapace made up of three anterior segments (Fused), the fourth thoracic segment being small, free and furnished in the females with a pair of dorsal plates overlapping below the genital segment are characters which place this form in the sub-family *Euryphorinæ* of the *Caligidæ*. Comparison with *Dissonus*, *Caligeria*, *Gloiopotes*, *Euryphorus*, *Elytrophora*, *Dysgamus* and *Alebion* of the *Euryphorinæ* make it amply clear that but for a superficial resemblance to *Gloiopotes* it is not related to any of the other genera mentioned above.

It can be distinguished from *Gloiopotes* by the very broad nature of the carapace, the presence of only three pairs of swimming legs, all of them biramose, the shape of the genital segment, the presence, more particularly, of wing-like expansions of the genital segment, the occurrence of two peculiar styliform appendages arising from the midventral line of the genital segment and the single jointed abdomen. It is therefore proposed to consider this parasite as a new genus to be named *Platyporinus* on account of the broad and flattened nature of the carapace and the genital segment and the new species is designated *Platyporinus alata*, on account of the wing-like expansions of the genital segment.

Analytical Key to the Genera of the Subfamily Euryphorinæ (from Wilson) incorporating the Present Genus Novo

1. Three thorax segments fused with the head; the fourth segment only free 2
1. Only the first thoracic segment fused with the head, the others free; no dorsal plates; all the swimming legs biramose, the rami three-jointed .. .. *Dissonus*, Wilson, 1906
2. One or more pairs of legs uniramose, the others biramose .. 3
2. All four pairs of swimming legs biramose 4
3. First legs only uniramose, their terminal claw curved and simple, setæ on anal laminæ short and non-plumose .. .. *Caligeria*, Dana, 1852
3. First and fourth legs uniramose; claws on first pair straight and three parted; setæ on anal laminæ long and plumose *Gloiopotes*, Steenstrup & Lutken, 1861
4. Exopod of the fourth legs three jointed, endopod two jointed 5
4. Both rami of fourth legs with the same number of joints .. 6
4. Fourth legs absent 6

5. Setæ on fourth legs plumose; first abdomen joint larger than second and covered with a dorsal wing or with two lateral wings .. .. .. *Euryphorus*, Milne Edwards, 1840

5. Setæ on fourth legs non-plumose; abdomen joints about the same size, without wings .. *Elytrophora*, Gerstæcker, 1853

6. Rami of fourth legs small, the two joints fused; the rami of first three pairs two jointed, without horny processes *Dysgamus*, Steenstrup & Lutken, 1861

6. Fourth legs rudimentary, hidden; exopods of first three pairs with horny processes .. .. *Alebion*, Kroyer, 1863

6. Fourth legs absent; exopods of the first three pairs of legs without horny processes .. *Platyporinus*, Gen. Novo

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STUDIES IN THE DROSERACEÆ

I. The Cytology of *D. indica* Linn., *D. Burmanni* Vahl., and *D. peltata* Sm. with Special Reference to Pollen Mitoses

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I. Introduction

The genus *Drosera*, according to Lloyd (1942), includes about 90 species. Of these only a few have been studied on a cytological basis. Rosenberg (1899, 1909) have given a cytological account of *D. rotundifolia*, *D. longifolia* and of the so-called natural hybrid *D. obovata*. Levine (1916) made a similar study on *D. rotundifolia*, *D. intermedia* and *D. filiformis*. Later work, in this field, by Behere (1929) and Rohweder (1937) deals mostly with the somatic mitoses of a few more species of *Drosera*, *Dionæa* and *Drosophyllum*. However, little is known on the cytology of the three Indian species—*D. indica*, *D. Burmanni* and *D. peltata* and it is with a view to provide some data, for these species, the present study was undertaken.

II. Materials and Methods

Materials for the present study were obtained partly from Tambaram and partly from Ootacamund. *D. indica* Linn. and *D. Burmanni* Vahl. were collected from the Seliur lake (Tambaram). These appeared, almost simultaneously, in the month of December and continued to grow till about the first week of March when the lake began to dry up. Although the two species grew side by side and in abundance, *D. Burmanni* was found to a larger extent than the other. *D. peltata* Sm. was collected near Ootacamund, at about an altitude of 7000′ and I am indebted to Prof. T. N. Venkatanathachari for the supply of this material for my investigation.

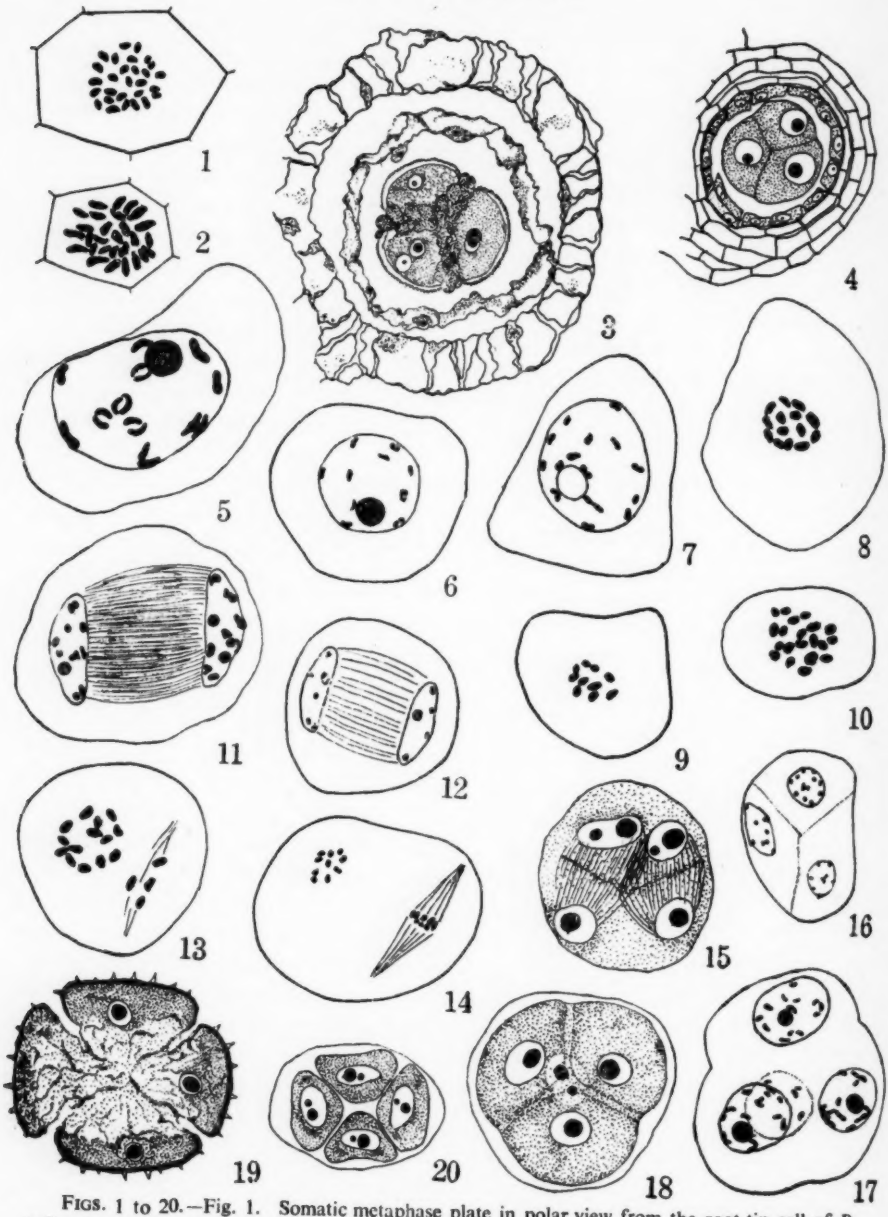
Root-tips and young flower buds of *D. indica* and *D. Burmanni* were pre-treated in Carnoy's fluid (6 parts by volume of absolute alcohol, 3 parts of chloroform and one of glacial acetic acid), washed in water for a few seconds and then fixed in Navaschin's fluid for a peroid of twenty four hours. The fixative was changed after the first two hours or so when it turned brown in colour. The nuclear fixative (corrosive sublimate 5 g., acetic acid 3 ml., and 30% alcohol 150 ml.) recommended by Suita (1937), was also employed, with success, especially in the study of post-meiotic stages in pollen grains. This is the only fixative used in the study of *D. peltata*. The usual schedule for dehydration and embedding was adopted and sections were cut at a thickness of 16 μ in the case of root-tips and 12–18 μ in the case of flower buds. Some of the preparations were stained in gentian violet and others in iron-alum hæmatoxylin. Drawings were made with a camera lucida, at bench level, using an apochromatic objective 1·25 in conjunction with different oculars.

III. Observations

(*a*) *Somatic mitosis.*—Poor development of the root system and the erratic nature of the divisions made the study of the root-tip mitosis a matter of some difficulty and only the root-tips of *D. indica* gave some results. Figs. 1 and 2 show the somatic chromosomes of this species, in polar view of metaphase, in cells of the root-tip and anther wall respectively. The chromosomes are rod-like and show little difference in size among themselves. The diploid number in both the instances was found to be $2n = 28$.

(*b*) *Meiosis.*—Meiotic studies were made in all the three species from diakinesis onwards. The pollen mother cells were examined mostly in T.S. of the anthers. Usually only three or four p.m.c.'s. (pollen mother cell) are seen in each loculus in a T.S., of the anthers, except in *D. peltata* where the number is still larger. In extreme cases, as in *D. Burmanni* and *D. indica* (Figs. 3 and 4), only one p.m.c. could be seen in each loculus in a T.S., and this almost fills up the whole cavity. Figs. 5, 6 and 7 show the p.m.c.'s. during synapsis in *D. indica*, *D. Burmanni* and *D. peltata* respectively. It could be seen from these, that the p.m.c. of *D. indica* is the largest of the three. This difference in size is also reflected at the pollen grain stage (compare Figs. 29, 30 and 31).

Thirteen bivalents (four of the ring-type and the rest of the rod-type) are observed in *D. indica* (Fig. 5). These are found to be considerably larger than those of the other two species. Two of the bivalents, namely, a rod and a ring are invariably found associated with the nucleolus. These are, probably, the nucleolar chromosomes. During this stage a prominent and peripherally placed nucleolus as well as a nuclear membrane are also visible.



FIGS. 1 to 20.—Fig. 1. Somatic metaphase plate in polar view from the root-tip cell of *D. indica* showing $2n = 28$ chromosomes (*ca.* × 1620). Fig. 2. The same as in Fig. 1 but the chromosomes are from a cell of the anther wall (*ca.* × 3240). Figs. 3 and 4. T.S. of an anther of *D.*

Burmanni and *D. indica* respectively showing only one quartet filling up the loculus (*ca.* × 740). Figs. 5, 6 and 7. Diakinesis in the p.m.c.'s, of *D. indica*, *D. Burmanni* and *D. peltata* respectively (*ca.* × 3240). (Note in *D. indica* the presence of both ring and rod bivalents ; in *D. Burmanni* all the ten bivalents appear as rods and in *D. peltata* one of the bivalents attached to the nucleolus is much longer than the rest. Figs. 8, 9 and 10. Metaphase I in *D. indica*, *D. Burmanni* and *D. peltata* showing $n=14$, $n=10$ and $n=20$ bivalents respectively (*ca.* × 3,240). Figs. 11 and 12. First telophase in *D. indica* and *D. Burmanni* respectively showing the spindle fibres and flattened nuclei (*ca.* × 3240). Figs. 13 and 14. Second metaphase in *D. indica* and *D. Burmanni* showing fourteen and ten univalents respectively (*ca.* × 3240). Fig. 15. Second telophase in *D. Burmanni* showing spindle fibres and cell-plate formation (*ca.* 3240). Fig. 16. Early stage in the organization of tetrads in *D. indica* showing cell-plate formation (*ca.* × 1620). Fig. 17. A later stage than 16 showing the beginnings of furrowing (× 3240). Fig. 18. Tetrad organization in *D. Burmanni* showing the beginnings of furrowing (*ca.* × 3240). Figs. 19 and 20. Tetrads of *D. indica* and *D. Burmanni* of the cruciate type (*ca.* × 1620).

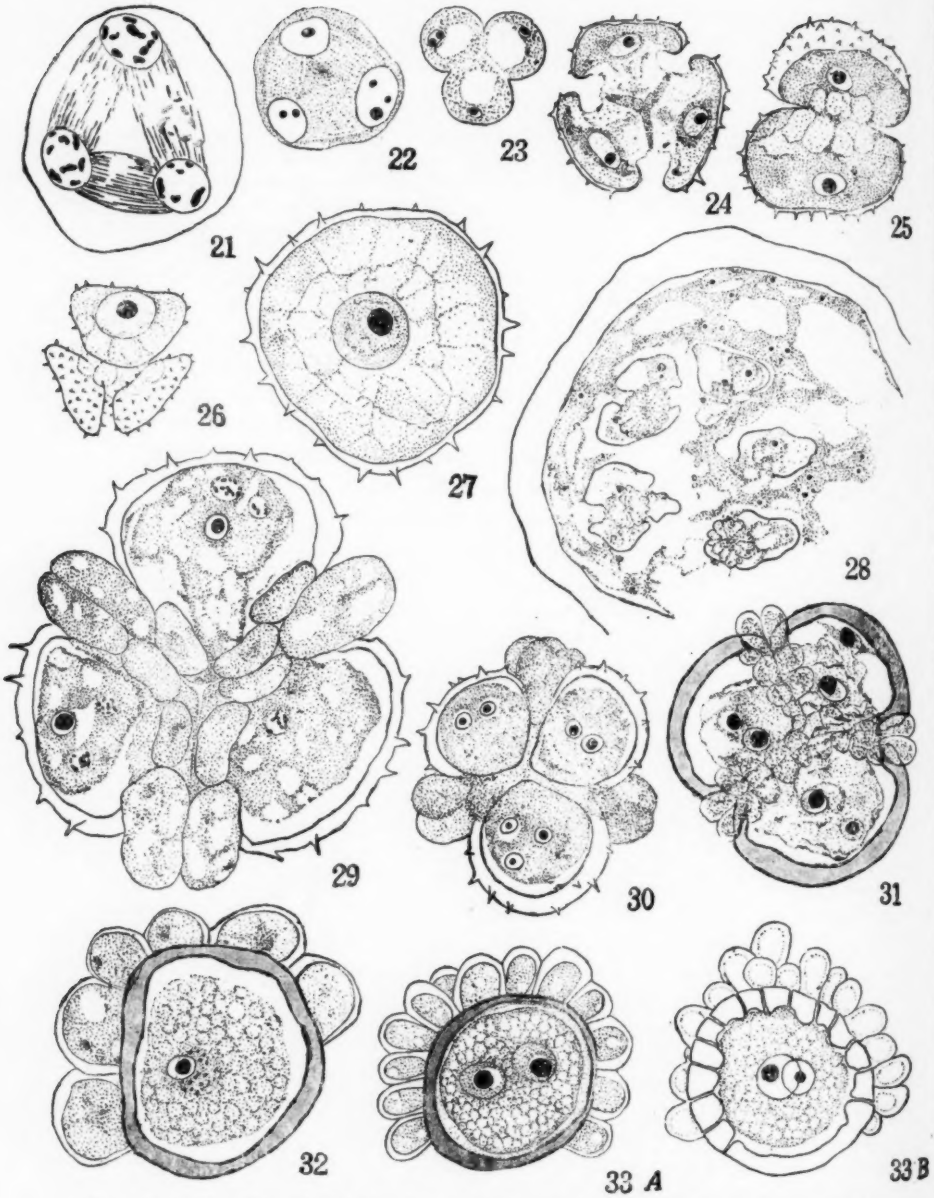
The p.m.c.'s. of *D. Burmanni* and *D. peltata* (Figs. 6 and 7) during diakinesis look similar except for a difference in the number of their bivalents. In both, the bivalents are found to be extremely small and rod-like, and exhibit the characteristic peripheral distribution. In each of these species a solitary nucleolus is also observed in a peripheral position. The bivalents, during diakinesis, could not be classified into rod and ring types. Almost all of them look rod-like. Ten bivalents are easily counted in *D. Burmanni* (Fig. 6). In *D. peltata* (Fig. 7) only 16 bivalents are seen although counts made during the first metaphase (polar view, Fig. 10) show unmistakable evidence of 20 bivalents for this species.

In *D. Burmanni* one of the bivalents is found regularly attached to the nucleolus (Fig. 6) while, in several of the p.m.c's. of *D. peltata* (Fig. 7) four bivalents are found attached to the nucleolus at different points. Of the four, one is found to be nearly twice as long as the rest.

Chromosome count is made in the polar view of the heterotypic equatorial plate and found to be $n = 14$ (Fig. 8), $n = 10$ (Fig. 9) and $n = 20$ (Fig. 10) for *D. indica*, *D. Burmanni* and *D. peltata* respectively. The haploid number $n = 14$ counted for *D. indica* corroborated the somatic complement $2n = 28$ determined from the cells of the root tip and anther wall. The few p.m.c.'s. available for study in *D. indica* and *D. peltata* indicate secondary pairing; but in the absence of more data nothing further can be said on this.

The first telophase and interphase are seen in *D. indica* (Fig. 11) and *D. Burmanni* (Fig. 12). As the interphase advances the two daughter nuclei lose their rotundity and become flattened. Each nucleus shows also the presence of a nucleolus.

In the second metaphase of *D. indica* and *D. Burmanni* (Figs. 13 and 14), which is quite normal, fourteen and ten chromosomes repectively are seen



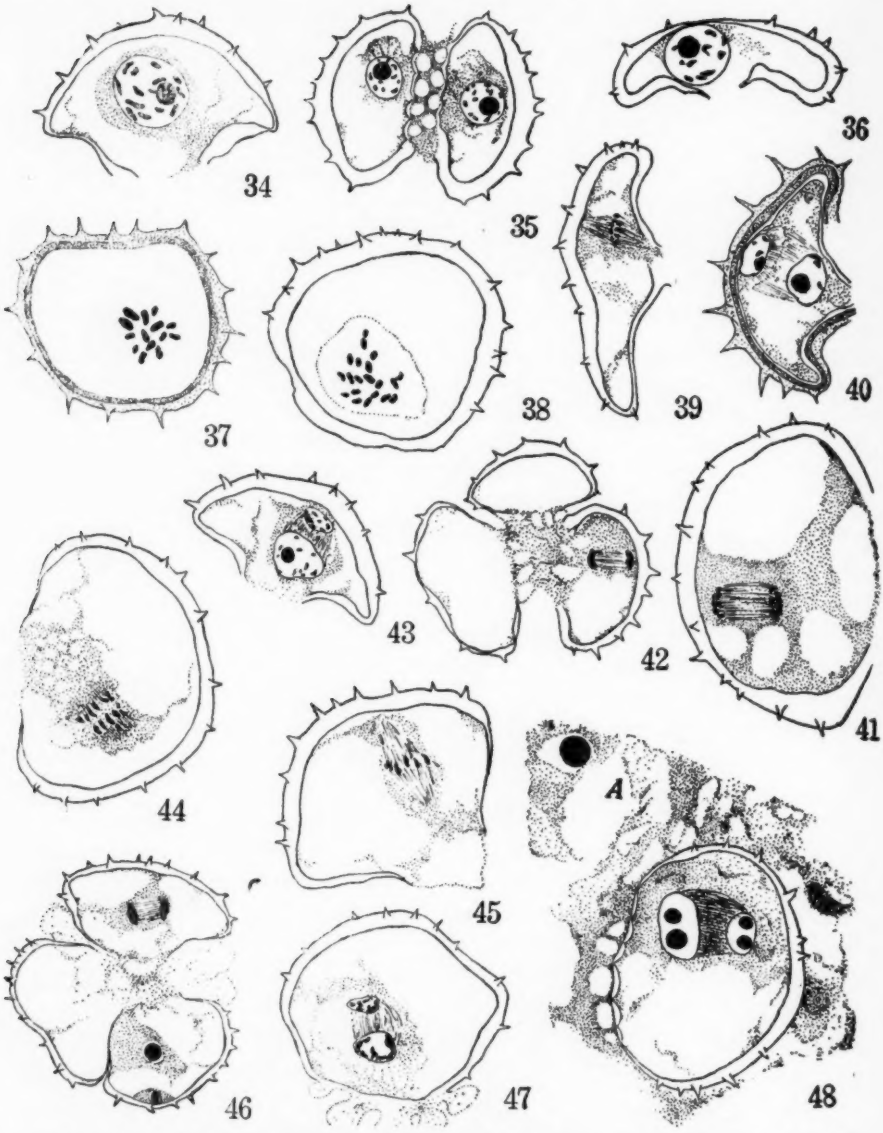
FIGS. 21 to 33.—Figs. 21 and 22. Tetrad organization in *D. indica* and *D. peltata* showing the persistence of the spindles (*ca.* × 3240). Fig. 23. A degenerating tetrad of *D. peltata* showing prominent vacuoles (*ca.* × 1620). Fig. 24. Degenerating pollen grains of *D. indica* presenting a flattened appearance (*ca.* × 820). Fig. 25. A stage in the degeneration of the associated

quartets of pollen grains in *D. indica*. Two have degenerated and out of the remaining two the one at the top is not likely to develop further (*ca.* 1620). Fig. 26. Quartets of *D. indica* with only one grain developing (*ca.* × 1620). Fig. 27. A single grain of *D. indica* which has developed at the expense of the rest of the associated quartets. Hence the big size (*ca.* × 1620). Fig. 28. T.S. anther of *D. peltata* showing pseudo-periplasmodium and degenerating quartets (*ca.* × 820). Figs. 29, 30 and 31. Pollen grains of *D. indica*, *D. peltata* and *D. Burmanni* respectively at the shedding stage. Note the three-celled condition in *D. indica*. In *D. peltata* only one of the associated quartets shows the three-celled condition while the remaining ones show only two cells—a case of non-simultaneous division. In *D. Burmanni* only two cells are seen. Figs. 32 and 33 *a*. Isolated pollen grains of *D. indica* and *D. Burmanni* showing the plaits on their dorsal side. Note that the two nuclei in *D. Burmanni* are of the same size (*ca.* × 1620). Fig. 33 *b*. Pollen grain of *D. Burmanni* showing numerous germ pores (*ca.* × 1620).

in polar view. These counts tally with the counts made during the first metaphase in these species. The two homotypic spindles lie parallel to each other. The second telophase in *D. Burmanni* (Fig. 15) is normal and shows the presence of phragmoplasts. Two of the daughter nuclei, on one side, regularly show the presence of an additional small nucleolus. Although phragmoplasts are formed during the early stages of tetrad organization, in both *D. Burmanni* and *D. indica* (Figs. 15, 16) quartets are developed only by the process of furrowing (Figs. 17, 18). In other words, the phragmoplast stage, in these, is only transitory. Second division in *D. peltata* was not available for study.

(*d*) *Tetrads.*—In all the three species the microspores are usually arranged tetrahedrally; the isobilateral arrangement was seen only rarely (*D. indica* and *D. Burmanni*, Figs. 19, 20). The microspores are organized simultaneously by the process of furrowing. In a number of cases (*D. indica* and *D. peltata*), the tetrad nuclei (Figs. 21, 22) are connected by spindle fibres. These are likely to have been formed anew as has been reported by Castetter (1926), Heimlich (1928) and Reeves (1930). Cooper (1935 *b*) has recorded a similar condition of the spindles connecting the tetrad nuclei. But, according to him, these spindles represent merely the remains of the heterotypic and the homotypic divisions. Some of the tetrads in *D. peltata* (Fig. 23) show a prominent central vacuole and a nucleus embedded in a thin peripheral cytoplasm. This is merely a symptom of degeneration as the tetrads undergoing normal development do not show vacuolization at such an early stage. But according to Levine (1916) the presence of such prominent vacuoles is a regular and normal feature in the p.m.c.'s., of *D. filiformis*.

Due to the degeneration of the sporocytes, at different stages of their development, there is a high rate of mortality, in the microsporangia of all the three species. Sometimes the entire contents of a loculus fail to develop. Degeneration is even more widespread at the tetrad stage. Out of the four microspores, one, two, three or in rare cases, all the four may perish (Figs.



FIGS. 34 to 48.—All figures have been drawn at an approximate magnification of × 1620 except Figs. 38, 41, 44, 45, 47 and 48 which have been drawn at a magnification of × 3240. Fig. 34. One of the associated pollen grains of *D. indica* showing its nucleus in prophase of the first pollen mitosis; 14 chromosomes can be counted. Fig. 35. Two of the associated pollen grains of *D. peltata* showing their nuclei in late prophase of the first pollen mitosis. Note the

position of the nuclei in relation to the vacuoles. Fig. 36. Pollen grain of *D. indica*, perhaps undergoing degeneration; hence the flattened appearance. The primary nucleus touches the dorsal and ventral sides of the grain. Fig. 37. A pollen grain of *D. indica* during metaphase of the first pollen mitosis. 14 Univalents of different sizes can be counted. Fig. 38. A pollen grain of *D. peltata* showing the primary nucleus in metaphase. 20 Univalents of about the same size are seen. Fig. 39. A degenerating pollen grain of *D. indica* in metaphase of the first pollen mitosis. The spindle is focussed at the poles. Fig. 40. First telophase of pollen mitosis in *D. indica* showing asymmetrical spindle, curved phragmoplast and the generative nucleus pressed against the wall of the pollen grain. Figs. 41 and 42. Early telophase of first pollen mitosis in *D. peltata* showing the distribution of the vacuoles in relation to the spindle. Fig. 43. First telophase of primary nucleus of *D. indica* ; note the size difference between the tube and generative nuclei. Figs. 44 and 45. Early and mid-anaphase of first pollen mitosis in *D. peltata*. In one case the spindle is focussed at both poles and in the other it is not. Note also the distribution of vacuoles. Fig. 46. Quartet of pollen grains of *D. peltata*. One of the grains show abnormal orientation of the first anaphasic spindle. In another quartet division is over and the generative and tube cells are seen. The third quartet is without its nucleus. Figs. 47 and 48. The primary nucleus of the pollen grain of *D. peltata* during telophase of first pollen mitosis. Note the asymmetric spindle, unequal size of the nuclei and their position right in the middle of the grain. Fig. 48 (*A*). A free primary pollen nucleus without a trace of the wall of the pollen grain ; probably a stage in degeneration.

24–27). When only a few of the quartets grow to maturity, the surviving ones are of a much larger size than usual. Consequently there is a certain amount of heteromorphism of the pollen grains due to nutrition, chiefly in *D. indica*.

In all the three species studied, the pollen grains remain associated in tetrads (see also Wodehouse, 1936 and Erdtman, 1943). This association continues even up to the time of opening of the anthers. However, during development some of the quartets undergo degeneration and their remains flow into the anther loculus sorrounding the unaffected grains. The remains of the degenerated grains gradually disappear. This is particularly noticeable in *D. peltata* (Fig. 28).

Although only a few functional pollen grains are found per anther, these are fairly large. The average diameter of the pollen in the three species is as follows:—*D. indica* 37 μ, *D. Burmanni* 31 μ and *D. peltata* 22 μ.

The pollen tetrads of the three species conform to the description of Wodehouse (1936) in that they resemble the pileus of certain amputated mushrooms. The distal or the ventral surface of the grain, in each of the species, is protected by a thick exine and a comparatively thin intine. In *D. indica* (Fig. 29) and *D. peltata* (Fig. 30), the exine is echinate, although the spines are less prominent in the latter. In *D. Burmanni* (Fig. 31) it is almost psilate or subechinate and the exine shows a fine saw-like contour. The proximal or the dorsal surface is comparatively thin and thrown into plaits which converge towards the centre of the tetrahedrally placed quartets.

Figs. 32, 33*a*, and 33*b* show these plaits, as seen in *D. indica* and *D. Burmanni* respectively. These look glandular.

Regarding germ-pores, these are confined mostly to the dorsal side of the grains and screened by the plaits. The number of germ-pores is 4, 6 and 14 respectively for *D. indica*, *D. peltata* and *D. Burmanni* (Figs. 58, 48 and 33*b*).

Further development of the tetrads into three-celled gametophytes is traced, chiefly, with reference to *D. indica* and *D. peltata* and are illustrated in Figs. 34–63.

(*d*) *First pollen mitosis.*—In the young condition the microspores (Figs. 18, 19, 26 and 27) are uninucleate and densely filled with cytoplasm. The nucleus, in these, is seen in a central position with reference to the grain. Further growth of the quartets is marked by an increase in their volume without a corresponding increase in their cytoplasmic contents. This leads to the formation of conspicuous vacuoles. Such vacuoles are formed in *D. indica* and *D. peltata* (Figs. 34, 35) even during the prophase of the first pollen mitosis. During this period the primary nucleus also becomes enlarged and takes up a position near the ventral side of the grain.

In a few tetrads of *D. indica* (Fig. 36) this enlargement of the primary nucleus, during prophase, is so marked that the nucleus is contiguous with the ventral and dorsal side of the tetrad. Such tetrads are somewhat flattened due to an increase in the diameter in the equatorial direction while their short axis remain more or less the same. It is quite probable that these do not develop further and may be a symptom of degeneration.

Although prominent vacuoles are formed inside the developing quartets, their nuclei are invariably sorrounded by cytoplasm. Figs. 34 and 35 represent the prophasic nuclei of *D. indica* and *D. peltata* respectively. In the former all the 14 chromosomes, of which one is nucleolar, are visible.

Metaphase chromosomes, in polar view, during first pollen mitosis, is found in *D. indica* (Fig. 37) and *D. peltata* (Fig. 38). In the former 14 chromosomes are counted. In the latter there are 20. These numbers give a further proof as to the correctness of the chromosome number, for these two species, determined during mitotic and meiotic divisions. The univalents, as seen in the first pollen mitosis, are small, rod-like and of different sizes, especially in *D. indica* (Fig. 37).

The plane of division of the primary nucleus, during first pollen mitosis, is invariably found to be at right angles to the long axis of the quartet (Figs. 39 and 45). In other words, the spindle fibres, during the first division, run

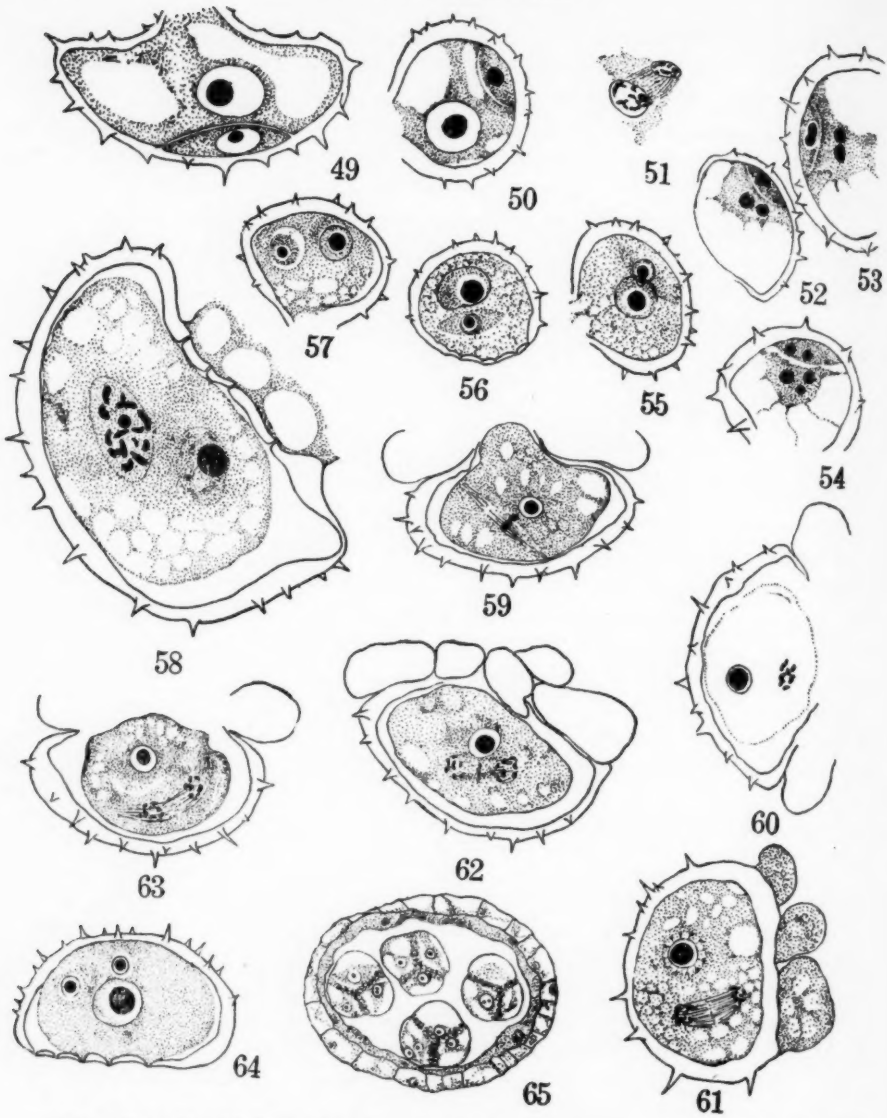
a course parallel to the short axis, occupying usually a position either to the right or to the left with reference to the centre of the grain. The position of the spindle, during this stage, is chiefly determined by the position of the major vacuoles. For example, in *D. indica* (Fig. 39) it is slightly to the right while in another quartet of the same species (Fig. 40) the spindle is seen right in the middle. In *D. peltata* also (Figs. 41, 42) a similar disposition of the spindle is seen. From these it is obvious that the orientation of the spindle, during the first post-meiotic division, depends upon the position of the major vacuoles.

Anaphase and telophase, in the first pollen mitosis are noticed in *D. indica* (Figs. 40, 43) and *D. peltata* (Figs. 41, 42, 44). During early anaphase the spindle is found to be symmetrical and focussed at the poles in *D. indica* (Fig. 39) and *D. peltata* (Fig. 45). Frequently, one of the poles of the spindle is found to be in contact with the intine on the ventral side of the grain while the other pole is almost free lying in the cytosome (Figs. 40-45).

In a few preparations of *D. indica* (Figs. 39, 40), the spindle is pretty short and the two poles are in contact with the intine on the ventral and dorsal side of the grain. A similar condition is noted for the prophasic nucleus also in the same species (Fig. 36). Probably the short spindle, in question, is only a further development of the nucleus in such degenerating pollen grains. The spindles observed in *D. peltata* appear to be of the 'elongated type' similar to what has been described by Johri and Nand (1935) in *Cuscuta reflexa* and Geitler (1935) in *Uvularia*. According to Darlington (1937) the anaphasic spindle is asymmetrical and this is a regular feature of pollen-grain mitosis. Such asymmetrical spindles have also been encountered by Bhaduri (1942) for the different species of *Tradescantia*.

It has been pointed out that in most cases the spindle lies parallel to the short axis of the pollen grain. However, in a few cases, a different orientation is observed. For example, in *D. peltata* (Fig. 46), one of the quartets shows its primary nucleus in telophase, and the spindle is orientated parallel to the long axis. According to Suita (1937) the orientation of the anaphasic spindle can be changed artificially. According to Darlington (1937) a change in the shape of the spindle is possible and is brought about by the modifying action of the centromeres on the spindle during anaphase.

The same figure (Fig. 46) also shows that all the quartets developing from a p.m.c., need not behave alike. Apart from the question of susceptibility to degeneration of the quartets, it is seen that the division of the primary nucleus, in these, is not synchronized. For, in one of the quartets



FIGS. 49 to 65.—All figures have been drawn at an approximate magnification of × 1620 diam., except Figs. 58 and 64 which have been drawn at a magnification of × 3240; Fig. 65 was drawn at a magnification of × 810. Fig. 49. Two-celled pollen grain of *D. indica*. Note the lens-shaped generative cell, its dense and homogeneous plasm. Note also the much larger and highly vacuolated tube cell. Fig. 50. One of the quartets of *D. peltata* at the end of the first pollen mtiosis. Note the lens-shaped generative cell and the disparity in size of the two nuclei

and nucleoli. Note also the absence of vacuoles in the plasm of the generative cell. Fig. 51. A case of primary nucleus of *D. indica* undergoing division in the absence of the microspore wall. Note that even in this condition the characteristic features of the division such as the asymmetric spindle and unequal daughter nuclei, etc., are seen. Figs. 52, 53 and 54. Two-celled male gametophytes of *D. peltata* showing fragmentation of the nucleoli of the vegetative and generative cells giving rise to three, four and five bodies which can easily be mistaken for 3, 4 and 5 nuclei. Fig. 55. Two-celled pollen grain of *D. peltata* showing the generative cell prior to migration into the tube cell. Fig. 56. The generative cell of *D. peltata* after migration. Note that the generative cell as a whole migrates and not merely its nucleus. Note also its spindle shape. Fig. 57. A later stage of the gametophyte of *D. peltata* compared to Fig. 56. Note that the spindle-like generative cell has now become spherical. Fig. 58. Second pollen mitosis in *D. indica*. The generative nucleus is seen in late prophase and 14 chromosomes and a nuceolus are seen. Note also the presence of 4 germ-pores. Figs. 59 and 60. Second pollen mitosis in *D. indica*. The generative nucleus is seen in the side and polar views of metaphase respectively. Note the presence of an attenuated spindle and different positions of the generative nucleus in relation to the grain and vegetative nucleus. Fig. 61. Early telophase of second pollen mitosis in *D. indica* showing the presence of phragmoplast, a definite spindle and vacuolated cytoplasm. Figs. 62 and 63. Anaphase or early telophase in the second pollen mitosis in *D. indica*; note the presence of cell plate in one and the crescent-shaped spindle in the other. In Fig. 62 the focussing of the spindle at the poles is not seen. Fig. 64. Three-celled pollen grain of *D. peltata* before dehiscence of the anther. Note the dense and homogeneous content of the gametophyte, the disparity in the size of the three nuclei and germ-pores. Fig. 65. T.S. of an anther of *D. Burmanni* showing the association of the pollen grains, and non-simultaneous division of the primary nucleus.

in question, the primary nucleus has not completed its first division while in the other a two-celled gametophyte is seen. Similar non-simultaneous pollen mitosis is found to prevail in the other two species also. Such a condition has been reported for *Crinum* sp. by Suita (1937).

Vacuolization referred to earlier, appears to reach its climax during anaphase of first pollen mitosis. A few prominent vacuoles are seen on either side or only on one side of the anaphasic spindle (Figs. 39, 40–48). Such prominent vacuoles are also seen in the quartet showing abnormal orientation of the spindle (Fig. 46). The vacuoles at this stage are characteristic. This will be treated more fully under discussion.

The organization of the first telophasic nuclei, in the first pollen mitosis, is seen in *D. indica* (Figs. 40,*43) and *D. peltata* (Figs. 41, 47, 48). During this stage, the spindle is markedly curved and become asymmetric although such things are not seen during anaphase. Of the two nuclei, the one destined to function as the generative nucleus is invariably smaller than the tube or vegetative nucleus and is cut off against the ventral side of the grain (Figs. 40, 43). These features are repeatedly seen in *D. indica* and *D. peltata*.

A few cases where the generative nucleus is not cut off against the ventral side of the grain have also been noted. In *D. peltata* (Figs. 47, 48) the primary nucleus during telophase is found to occupy a position almost in the middle of the grain. Here the generative nucleus is not contiguous with

the intine on the ventral side as is usually the case. Even in such grains, the characteristic size difference between the two daughter nuclei, mentioned previously, is noticed. It is also seen that the smaller of the two nuclei is found towards the ventral side of the grain. This would suggest the possibility of a definite polarity for the anaphasic spindle during first pollen mitosis.

There is good evidence that phragmoplast is formed during telophase of the first pollen mitosis. This is actually seen in *D. indica* (Fig. 40). The phragmoplast, as has been pointed out by Wulff and Maheshwari (1938) " During its increase in width the outer fibres are not directed towards the vegetative nucleus but abruptly towards the wall of the microspore, thus giving rise to the lens-shaped generative cell." Rosenberg (1909) has also figured and described such a condition in *D. obovata*. Phragmoplast is not actually seen either in *D. Burmanni* or *D. peltata*. However, the presence of ' lens-shaped ' generative cell in these also would lend a certain amount of indirect evidence for its occurrence.

At the end of first telophase, of the first pollen mitosis, two very unequal cells are cut off as seen in *D. indica* (Fig. 49) and *D. peltata* (Fig. 50). The larger of the two is the tube cell and the other the generative cell. The two are separated by a convex clear space (Figs. 49, 50). In any case, all the three species of *Drosera* furnish evidence to show that a distinct generative cell is cut off against the intine on the ventral side of the grain. Thus, the first pollen mitosis is completed. The position and lenticular shape seem to be very characteristic of the generative cell in this genus, barring such exceptions as have been referred to in *D. peltata* (Figs. 47, 48).

The generative and tube cells, in the three species, show a few differences. The nucleus of the tube cell is usually larger than that of the generative cell. The respective nucleoli, too, share this feature. Usually the nuclei in question show only one nucleolus per nucleus, although in *D. peltata* two or more are found. This will be treated fully further down.

Wulff and Maheshwari (1938) have stated that even with Haidenhein's hæmatoxylin one ought to see a difference in the staining capacity of the plasm of the tube and generative cells. Several previous workers have noted that the plasm of the generative cell stains less compared to that of the tube cell. In some cases the plasm of the generative cell is not stained at all. In *D. indica* the plasm of the generative cell stains a shade deeper than that of the tube cell.

In a few degenerated quartets of *D. peltata* (Fig. 69 A) both the exine and intine are obliterated leaving intact only the primary nucleus embedded

in a little of the cytoplasm. A similar case is found in *D. indica* also (Fig. 51). Here, the primary nucleus, in telophase, is found to persist while the other parts of the quartet are not present. That the nucleus in question is the primary nucleus of a quartet is inferred from the asymmetrical nature of the spindle as well as the unequal daughter nuclei formed (compare Figs. 51, 48 and 47). It appears as though that this dividing nucleus exists in a free state similar to the endosperm nuclei before wall formation. The point of similarity alludes only to their mitotic activity in the absence of cell wall.

(*e*) *Abnormality in the pollen grain of* D. peltata.—The abnormality is chiefly noticed in the two-celled male gametophytes of *D. peltata*. Normal 2-celled gametophyte of this species (Fig. 50) shows two nuclei each with a prominent nucleolus. The abnormal ones, on the other hand, show more than two nucleoli. In Fig. 52, the nucleus of the tube cell shows two nucleoli instead of one. The nucleolus in the generative cell shows no change except that it has become slightly rod-like. In Fig. 53, this rod-like nucleolus of the generative cell becomes constricted in the middle; probably it is about to divide. In Fig. 54 the nucleolus in question has divided into two and in the tube cell there are three nucleoli. Several preparations of *D. peltata* show these features regularly. It is possible that under certain conditions, the nucleoli concerned undergo fragmentation and produce three, four, five or even six bodies in all. The resultant nucleoli are neither spherical nor of uniform size. Perhaps, such nucleolar fragmentation is not a feature of normal gametophytes but only of those undergoing deterioration. Swamy (1941) has also recorded such nucleolar fragmentation in the vegetative nucleus in the pollen tube of *Cymbidium bicolor*.

Fragmentation of the nucleolus, in the vegetative and generative nuclei, does not occur simultaneously. This, sometimes, leads to the formation of three deeply staining bodies (Fig. 52) and in this condition can easily be mistaken for a three-celled gametophyte. However, the three-celled gametophyte of *D. peltata* (Fig. 30) can easily be distinguished from a two-celled one containing merely three nucleoli (Fig. 52). The three-celled male gametophyte becomes deeply stained than the two-celled one. Further, in the three-celled condition the vacuoles seen during earlier stages, disappear and the gamethopyte becomes packed with food reserves.

The question arises as to the real nature of these darkly staining bodies (Figs. 52, 53, 54) which are interpreted as nucleoli. If these are not nucleoli, then they should be in the nature of nuclei. In the latter case, *D. peltata* shows not only a three-celled gametophyte but also gametophytes contain-

ing four, five and even six cells. No doubt, a few stray cases of male gametophytes containing more than three nuclei, at the time of shedding, have been reported by Chamberlain (1897), Johri (1935) and Venkateswaralu (1947). But such cases are few and far between. On the other hand, nucleolar fragmentation is much more common.

Johri (1935) in *Cuscuta reflexa*, has cited an instance where the generative nucleus of the pollen grain " Was in a process of fragmentation into two nuclei ". A reference to his diagram suggests to me that it can be regarded more a case of fragmentation of the nucleolus than of the nucleus. Venkateswaralu (1947) in *Wikstræmia canescens*, has figured and described an abnormal pollen grain showing two generative nuclei and two vegetative nuclei. The nuclear nature of two of these bodies, especially those referred to as vegetative and generative nuclei, does not suggest itself from his diagram. These two bodies look more like nucleolar fragments. If this is admitted, then the so-called abnormal grain would become a normal three-celled grain consisting of two sperm cells and a tube cell in which the nucleolus of the tube nucleus has fragmented prior to degeneration. Such a degeneration of the tube nucleus, without affecting the two male cells, is quite possible and is in accordance with Suito's observations. According to Suito (1937), the further development of the male gametophyte after the three-celled stage in species of *Crinum* is invariably marked by the degeneration of the tube nucleus.

After the first pollen mitosis, there is an increase in the cytoplasmic contents of the tube cell. The prominent vacuoles, so characteristic of the first pollen mitosis, disappear and the plasm of the tube cell look frothy and include several extremely small vacuoles. Perhaps, food materials in the form of cytoplasmic inclusions are accumulated (Figs. 55, 56, 57). Such changes in the contents of the tube cell have been recorded by Bahadur Singh (1934), Cooper (1935) and Kajale (1940) for different materials. Side by side with these changes, in the plasm of the tube cell, the generative cell assumes a somewhat spindle-like form and at the same time begins to change its characteristic position. All these changes set in when the gametophyte gets ready for the second pollen mitosis.

(*f*) *Second pollen mitosis.*—The first indication of the oncoming change in the generative cell is foreshadowed by the gradual disappearance of the convex space separating the plasm of the tube cell from that of the generative cell. It appears as though that the two plasm coalesce thereby setting free the generative nucleus to wander in the now common cytoplast. The different positions taken up by the generative nucleus, as it wanders in the common cytoplast, is seen in *D. indica* (Figs. 55–63).

There is a controversy as to whether the generative cell or its nucleus that wanders in the general cytoplasm. Finn (1925) refers to the withdrawal of the generative cell from the pollen grain wall in *Asclepias Cornuti*. He has also stated that during the early stages of withdrawal the two are connected by a thin band of cytoplasm. Maheshwari (1934) has stated that "A wall is laid down between them at first but later it dissolves leaving the two nuclei free in the cytoplasm of the pollen grain." Kausik (1939) has referred to the generative cell lying towards one side and the plasm of the sheath becoming obscure at the time of dehiscence. Wulff and Maheshwari (1938) have discussed this point and the consensus of opinion seems to favour the view that a plasm is found around the wandering generative nucleus. Its absence is attributed only to faulty technique. So far as the *Drosera* species are concerned, the plasm is actually seen in *D. peltata* (Figs. 55, 56, 57), which becomes indistinguishable prior to the second mitosis. In *D. indica* (Figs. 58–63) the generative nucleus does not show a sheath at the time of division.

There are also questions like the position of the generative nucleus during second pollen mitosis, its shape, etc. Observations made on *D. indica* and *D. peltata* show that no special position is taken up by the generative nucleus during division. This inference is drawn because of the different positions taken up by the generative nucleus (Figs. 58–63) before division. According to Sharp (1934) the generative nucleus invariably takes up a position near the wall of the grain.

Regarding its shape, Finn (1925) has stated for *Asclepias Cornuti* that prior to its migration it becomes spindle-like and later on the spindle becomes further elongated "With much extended tail-like ends". That it becomes spindle-like is seen in a few preparations of *D. peltata* (Fig. 56). The spindle-like generative nucleus becomes spherical at a later stage (Fig. 57). Therefore, prior to the second division in the pollen of *D. peltata*, both the tube nucleus and the generative nucleus become spherical. This is true also in *D. Burmanni* (Figs. 33 *a* & *b*). Such a spherical generative nucleus has also been recorded by Swamy (1943) in *Eulophia*. It must be said that most of these observations were made in T.S. of anthers and therefore the spindle-like form of the generative cell cannot be observed. As Cooper (1935) has pointed out, the spindle-shaped cell would appear only circular or spherical when it is cut transversely and oval in oblique sections.

In *D. Burmanni* (Fig. 33) the two nuclei (tube and generative) are more or less alike. Presumably the nucleus of the generative cell increases in size just before division. The generative nucleus in *D. indica* and *D. peltata* divides

within the grain before shedding takes place. A few stages in the division of the generative nucleus of *D. indica* are shown in Figs. 50–55.

Fig. 58 shows the generative nucleus of *D. indica* in late prophase of second pollen mitosis. The nuclear membrane is hardly noticeable and the condensation of the chromatin material has taken place to such an extent that the individual chromosomes are easily counted. Fourteen curved and rod-like chromosomes are made out, at this stage, together with a spherical nucleolus. Finn (1925) has also recorded a similar condition in *Asclepias Cornuti*. In his own words "The generative nucleus, with still prominent outlines, includes a slightly stained nucleolus and twelve already individualized chromosomes appearing in most cases as short and somewhat curved rods." A parallel instance of very individualized chromosomes similar to metaphase ones has also been recorded in the generative nucleus lying within the pollen tube of *Impatiens balsamina* by Raghavan, Venkatasubban and Wulff (1937). The individualized chromosomes, in question, in *D. indica* give one more opportunity to verify that the haploid number for this species is $n = 14$.

Figs. 59 and 60 show the generative nucleus during metaphase in side and polar view respectively. In the former, a well-defined, symmetrical and obliquely placed spindle, pointed at the poles, is seen. The spindle is so much elongated that it touches the intine on the dorsal and ventral sides of the grain. Opinion is not unanimous as to the focussing of the spindle at the poles. Finn (1925) in a species of *Asclepias* found no such focussed spindle while Gager (1902), in the same material was able to observe a spindle focussed at the poles. Figs. 59 and 60 also show that the generative nucleus, at the time of division, can remain near the ventral or dorsal side of the grain. Further, the dividing nucleus is placed near the tube nucleus (Figs. 59, 62) or away from it (Figs. 60, 61 and 63).

During late anaphase of the second pollen mitosis, the spindle becomes elongated and sickle-shaped (Fig. 63). Such a spindle, however, is not seen in Figs. 61 and 62. The spindles, in these figures, are not focussed. Perhaps, they represent only the middle portion of spindles concerned. The spindle in *D. indica* appears to be part intra-nuclear and part extra-nuclear.

A regular cell plate, in the equatorial region of the spindle, is seen in *D. indica* (Figs. 61, 62) during telophase of the second pollen mitosis. This is in accordance with the findings of Cooper (1935 *a*, 1936). On the other hand, Wulff (1933) regards the cell plate as purely transitory and that cytokinesis is due to furrowing. This could not be verified in *D. indica* as stages beyond the second telophase were not available for study.

(*g*) *Mature pollen grain.*—The pollen grains of *D. indica* (Fig. 29) and *D. peltata* (Figs. 30 and 64) reach a three-celled stage only just before the shedding of the grains. Only a two-celled condition is found in *D. Burmanni* (Fig. 31). Narasimhachar (1949) has also recorded the same for this species. It is also likely that the pollen grains in *D. Burmanni* are three-celled like those of *D. indica* and *D. peltata* but the nuclei are obscured by the cytoplasmic inclusions present within the grain. The two sperm cells are found to be spherical and not elongated. At about this time the pollen grains become densely packed up with food reserves and stain more intensely than before. This is particularly so in *D. Burmanni.* Kajale (1940) has also noted similar changes taking place in the maturing pollen grains of the Amarantaceæ.

The three-celled stage is not attained simultaneously in all the quartets. For instance, in *D. peltata* (Fig. 30) only one of the associated quartets is in the three-celled condition while the other two are only two-celled. Apparently the generative cell has a long period of rest before it divides to give rise to two male cells. *D. Burmanni* also shows such non-simultaneous division (Fig. 65).

IV. Discussion

Out of about 90 species of *Drosera*, chromosome numbers are available for only a dozen species. The numbers, as given by Gaiser (1930) and by Darlington and Janaki Ammal (1945), range from $2n = 20$ to $2n = 80$. A diploid number of $2n = 20$ has been recorded for *D. filiformis*, *D. pygmea*, *D. rotundifolia*, *D. intermedia* and *D. Burmanni*. A diploid number of 40 has been found in *D. capensis*, *D. longifolia*, *D. anglica* and *D. peltata*. In *D. cistifolia* and *D. spathulata* the diploid numbers are found to be $2n = 60$ and 80 respectively. Thus, the above species of *Drosera* provide a typical polyploid series of $2n = 20$, 40, 60 and 80. These numbers suggest that in the speciation of *Drosera*, polyploidy has played some part.

Other numbers, besides those cited above, have also been recorded. *D. indica* shows a diploid number of $2n = 28$. Rosenberg (1909) has recorded $2n = 30$, for *D. obovata*. *D. obovata* is considered to be a natural hybrid between *D. longifolia* ($2n = 40$) and *D. rotundifolia* ($2n = 20$). The cytology of *D. obovata*, investigated by Rosenberg (1909), clearly indicates the possibility of hybridization also as a factor concerned in speciation in the genus. Such a natural hybrid as *D. obovata* might have become stabilized through amphidiploidy and one could predict the possibility of a second polyploid series, with diploid number like $2n = 30$, 60, 90, etc.

The occurrence of such numbers as $2x = 28$, and 12 in *D. indica* and *Dionæa muscipula* respectively, would suggest that aneuploidy too has acted

in the formation of genera and species in the Droseraceæ. It appears, therefore, that polyploidy, aneuploidy, hybridization, etc., are the chief factors concerned in the evolution of this family. Further, the chromosome numbers available for the different genera and species would suggest 10 as the basic number for the Droseraceæ. This is in accordance with the views of Behere (1929).

The nutrition of the sporogenous cells, till they develop into three-celled male gametophytes, also presents some interesting points. The tapetum is involved during the earlier stages. That material transfer takes place from the tapetal cells to the p.m.c.'s., is inferred from the appearance of intrusive tapetum and the formation of pseudo-periplasmodium.

After the depletion of this source of food, some of the pollen grains degenerate and their material remains are resorbed by the survivors. This generally takes place during the first and second pollen mitosis when the grains become fully charged with food materials. Perhaps, this is one of the reasons why the pollen tetrads remain associated even at the time of dehiscence. A point in favour of this view is gained if it is remembered that seldom do all the four quartets develop into three-celled gametophytes. Although this mode of association of the developing pollen grains would appear abnormal, it is the most normal thing in the cases of the four megaspores that are formed during the development of the embryo-sac. Some similarity also exists between the nutrition of the quartet at the expenses of other associated quartets and the way in which the functioning megaspore grows at the expense of the associated potential megaspores.

Aggregation of pollen grains has been observed not only in the different species of *Drosera* but also in other genera like *Dionæa* (Smith, 1929), *Typha latifolia*, *Junicoides campestre*, *Drimys* sp. (Wodehouse, 1935), *Condylocarpus* (Rendle, 1925), members of the family Asclepiadaceæ and Orchidaceæ. Such compound grains might have arisen as a nutritional device as is the case in *Drosera*, *Dionæa*, etc., or with a biological end in view as in the families Asclepiadaceæ and Orchidaceæ. It is also evident that such compound grains have originated several times independently in course of time since such grains are found in unrelated families.

The first pollen mitosis in *D. peltata* and *D. indica* also calls for a few remarks. In these species the spindle occupies a characteristic position. One pole is invariably free while the other rests against the intine on the ventral side of the grain, the orientation of the spindle being at right angles to the long axis. A few exceptions were, however, found in *D. peltata*. In one case (Figs. 41, 42) the dividing primary anaphasic nucleus is found in

the centre of the grain. In another (Fig. 46) the spindle is placed in a direction parallel to the long axis of the grain and at the same time the poles are free. A careful study of the position of the spindles, during this stage, shows that they are definitely placed with reference to the major vacuoles. In other words, the disposition of the spindle during the first pollen mitosis depends on the distribution of the cytoplasm and vacuoles within the quartet. However, Geitler (1935) has claimed a fixed position for the spindles in the microspore in every species.

The asymmetrical spindle formed during the first post-meiotic division presents something of interest. Such a spindle is especially characteristic of late telophase in both *D. indica* and *D. peltata*. Darlington (1937) has explained this asymmetry as follows: "The cell-wall stops one group of chromosomes from moving further in mid-anaphase, but the stretching of the spindle continues to push the other group, which is still seen in the characteristic attitude of movement." This explanation would hold good for those cases where one of the daughter nuclei is regularly cut off against the intine of the grain. But some other explanation will have to be found for cases where the nuclear division takes place right in the middle of the cell as shown in Figs. 47 and 48. Here, the cell wall does not stop one group of chromosomes from moving further. Even under these conditions the same asymmetry and curvature of the spindle are observed.

Further, the two daughter nuclei formed at the end of the first pollen mitosis in *D. indica* and *D. peltata* are markedly different in size, a fact recorded by several investigators. According to Geitler (1935), this size difference is due to unequal distribution of the karyolymph. The first pollen mitosis is a characteristic division resulting in two structurally and functionally different nuclei. That they are different, can be seen not only from their difference in size and staining qualities but also from their subsequent behaviour. The vegetative nucleus disintegrates and is dissolved by the ferments produced by the grain (Suita, 1937) while the generative nucleus remains unaffected. Over and above this there is the question of production of sperms only from the generative nucleus.

The occurrence of primary nucleus of pollen grains, in different stages of division, in the absence of a cell wall has been referred to already in *D. indica* and *D. peltata*. The free nuclei in question are found in different stages of division and the one in telophase is shown in Fig. 51. Even this nucleus shows the characteristic features of first pollen mitosis such as the asymmetrical spindle, unequal daughter nuclei, etc. The question arises as to whether the mitotic changes have taken place before or after

the disappearance of the wall of the quartet which once housed the nucleus concerned. In other words can the primary nucleus behave independently of the wall of the quartet surrounding it? The following sequence of events appears probable to me. At first the wall of the quartet, undergoing degeneration, gets quickly dissolved due to some enzyme secreted by the cytoplasm. Probably this enzyme has no action at all on the primary nucleus. Now this primary nucleus, which is surrounded by a little cytoplasm, undergoes the usual mitotic cycle.

Second pollen mitosis, observed chiefly with reference to a few preparations of *D. indica* (Figs. 59–63) is quite normal and did not show any deviation from normal mitosis. The metaphase chromosomes are scattered in the equatorial plate. A distinct spindle and phragmoplast are also seen. These points are specially mentioned since the dividing generative nucleus inside the pollen tube usually show none of these features. In the different species of *Impatiens* it has been recorded by Wulff (1933) and Raghavan, Venkatasubban and Wulff (1939) that the metaphase chromosomes of the generative nucleus, in the pollen tube are placed more or less in a linear row. Further, in the same material, neither spindle fibres nor phragmoplasts have been recorded. These differences in the behaviour of the generative nucleus during division within the microspore and pollen tube have been partly explained on the basis of spatial limitations. No doubt, the characteristic disposition of the metaphase chromosomes, in the pollen tube, can be understood as due to want of more space; but it is rather difficult to understand how such spatial consideration can suppress spindle and phragmoplast formations in the dividing generative nucleus. On the whole, one cannot but reflect on this varied behaviour of one and the same nucleus although under different circumstances.

V. Summary

Three species of *Drosera—D. indica*, *D. Burmanni* and *D. peltata*, were ubjected to a cytological study.

A diploid chromosome number of $2n = 28$ was counted, for the first time, during mitosis in the root-tip and anther wall cells of *D. indica*.

Meiotic stages, from diakinesis onwards, were traced, in all the three species and found to be normal. Division of the p.m.c. was found to be of the simultaneous type. The following haploid numbers were determined for the first time during heterotypic and homotypic divisions:—*D. indica*, $n = 14$; *D. Burmanni*, $n = 10$ and *D. peltata*, $n = 20$.

Pollen tetrads were formed by a process of furrowing and in all the three species remained associated till they were discharged from the anther. Pollen mortality was found to be rather high.

Post-meiotic changes in the pollen grains of *D. indica* and *D. peltata* have been studied in detail. Division of the microspore nucleus, in a few cases, was found to be of the non-simultaneous type. In the metaphase (polar view) of the first pollen mitosis of *D. indica* and *D. peltata*, 14 and 20 univalents respectively were counted. The details of anaphase and telophase of first pollen mitosis have been included and discussed. A generative cell is formed at the end of the first pollen mitosis in all the three species.

A few cases of nuclear fragmentation and abnormal orientation of the anaphasic spindle are described under *D. peltata*.

Second pollen mitosis, studied chiefly with reference to *D. indica* proved to be normal. A well-defined spindle and cell plate were distinguished.

Pollen grains in *D. indica* and *D. peltata* at the time of shedding were found to be three-celled. In *D. Burmanni*, only two cells could be made out.

The role of polyploidy, aneuploidy, hybridization and amphidiploidy have been discussed in relation to the evolution of different genera and species in the Droseraceæ.

The basic number ten has been suggested for the family.

In conclusion, I would like to express my indebtedness to Dr. P. Maheshwari, for having suggested this problem, for kindly going through the manuscript and for his valuable suggestions. I would also like to thank Dr. T. S. Sadasivan for the valuable help received during the progress of this work.

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ON SOME EEL EGGS AND LARVÆ FROM THE GULF OF MANAAR*

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Introduction

Though much attention has been devoted to the elucidation of the biology of the eels, the records of their eggs are very few and, strangely enough, even the egg of the European eel, *Anguilla vulgaris*, which was the subject of intensive investigation, remains unknown to Science. The first account of eel eggs was by Raffaele (1888) who studied the early development of five types of unidentified eggs from the Bay of Naples. This was followed by Eigenmann's (1901) investigation on the development of an eel egg resembling Raffaele's "Species No. 6" collected 30 miles south of South Shoal and assigned by him to the Conger eel. Boeke (1903) described nine types of eel eggs and larvæ from the Naples Bay which included the types studied by Raffaele. Schmidt (1913) described the eggs of the Mediterranean Conger, *Muræna helena*. He also collected the eggs of *Nettastoma melanurum* from the Balearic and Tyrrhenian Seas and identified Raffaele's "Species No. 7" and Boeke's "Muræna No. 3" as that of *Ophichthys hispanus* and Boeke's "Muræna No. 2" as that of *Ophichthys serpens*. Fish (1927) gave an account of the egg and larvæ of the American eel, *Anguilla rostrata*, collected 10 miles south-west of Bermuda Island. Recently Delsman (1933) described a few eggs and larvæ of eels obtained from the Java Sea.

The only record of eel eggs from Indian waters is by Aiyar, Unny and Varkey (1944, Abstract) from the Madras Coast.

The present study is based on a collection of twelve eel eggs and six larvæ obtained from the surface plankton collections made on the 12th (Lat. 8° 44′ 54″ N., Long. 78° 19′ 5″ E.) and the 13th (Lat. 8° 44′ 54″ N., Long. 78° 20′ 15″ E.) April, 1949 at Tholayiram Paar, one of the pearl banks in the Gulf of Manaar situated 11 miles off Tuticorin Bay with a depth of

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8 to 11 fathoms. The sea bottom of this Paar consists of rocky outcrops supporting varied types of corals, sponges, sea weeds and other forms. The collections were made from 9–45 to 10 a.m. with a three-fourth meter organdie townet from M.F.V. " Gohar Khaleeli " at a speed of 2 knots. The hydrological conditions at the time of collection were:—

		12–4–1949	13–4–1949
Temperature	..	29·79°C	29·65° C.
Specific Gravity	..	1·024	1·024
Salinity	..	not available	33·87%
pH	..	,,	8·5

A detailed study of the eggs and larvæ could not be undertaken on board for want of facilities for such work and on account of rough weather at the time. The material was, therefore, fixed in 5% formalin and examined in the Laboratory later. The eggs had all the distinctive characters of eel eggs, *viz.*, very large size, large perivitelline space and segmented yolk, and the characters of the egg and the developing embryo and the presence or absence of oil globule showed that they belonged to three distinct species of eels. The two types of eel larvæ present in the collection cannot definitely be said to belong to the eggs described here. In comparison with the larvæ described by Delsman they appear to be three or four days old.

Egg I

This eel egg seems to be common in the locality judging from the number (eight) present in the collection showing the embryo in different stages of development. The egg is transparent and spherical having a diameter of 2·5 mm. In the earliest stage obtained, only the indication of the formation of the embryo is seen as a streak (Fig. 1). The yolk is spherical with a diameter of 1·4 mm. and shows the vacuolated or segmented nature very clearly. The perivitelline space is wide. Some eggs show the embryos in a fairly advanced condition with well formed eyes, otocyst, heart and œsophageal pouch (Fig. 2). The myotomes have differentiated of which 82 can be counted in this stage. The caudal end of the embryo is recurved and free from the yolk. The yolk mass is slightly elongated.

Egg II

This is the largest eel egg in the collection measuring 3·2 mm. in diameter. It is perfectly transparent and spherical with a large perivitelline space. In the earliest stage obtained, the embryo is small and occupies about half the circumference of the yolk (Fig. 3). The yolk mass is spherical and clearly vacuolated. The eyes, otocyst, heart and œsophageal pouch

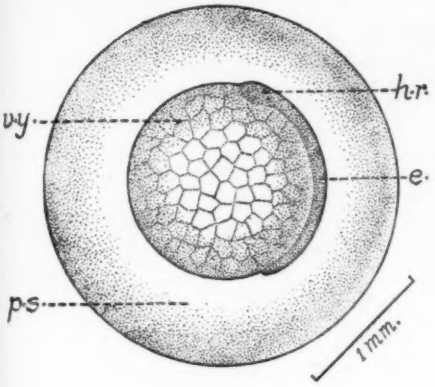

FIG. 1

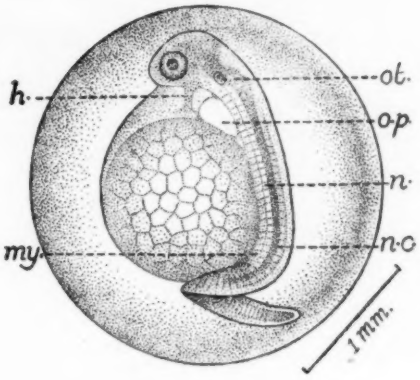

FIG. 2

Figs. 1 & 2. Two stages in the development of Egg I

have formed and 42 myotomes are seen faintly in this stage. In the next stage, the embryo is larger and completely encircles the elongate yolk mass with the caudal portion free from it (Fig. 4). About 105 myotomes are present in the embryo with the vent situated behind the yolk opposite the 73rd myotome. In a more advanced stage, the embryo is longer with the widened caudal portion showing distinct fin-folds (Fig. 5). More myotomes are formed in this stage and their number is 124 with the vent occupying the same position as in the previous stage. The yolk mass has

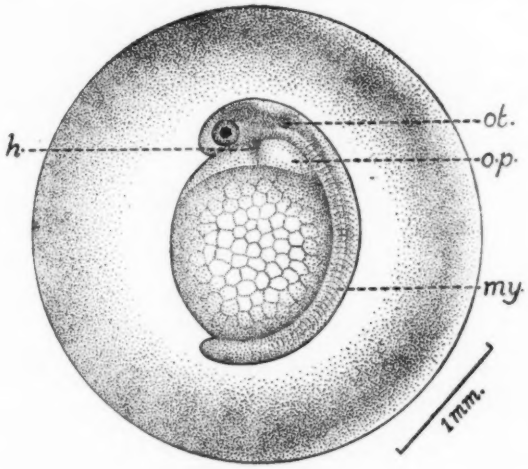

FIG. 3

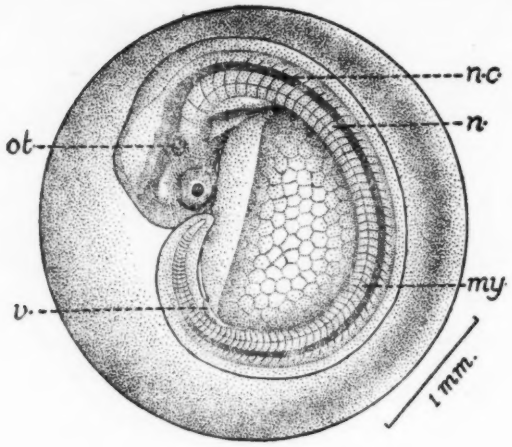

FIG. 4

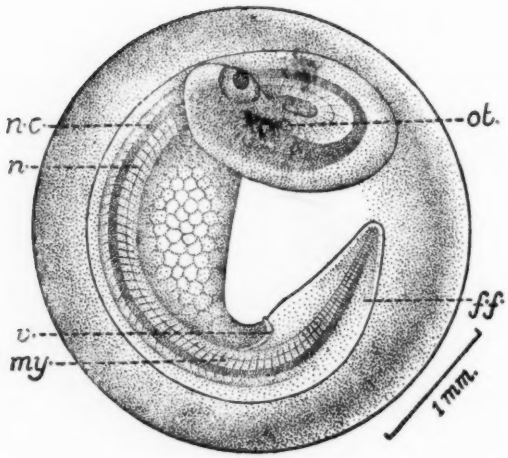

FIG. 5

Figs. 3 to 5. Three stages in the development of Egg II

assumed an elongated fusiform shape and is confined to the posterior portion of the preanal region.

EGG III

There is a single egg of this type, 2·5 mm. in diameter, in the collection. It is transparent and spherical (Fig. 6). The spherical vacuolated yolk is

1 mm. in diameter. A single oblong oil globule is present peripherally in the yolk. The embryo is in an advanced stage of development with the long caudal portion free from the yolk. The eyes, otocyst, heart and œsophageal pouch have formed. 63 myotomes have already differentiated in this stage.

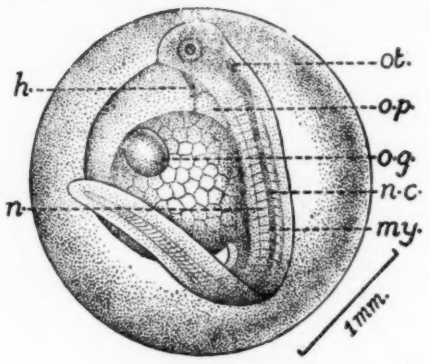

Fig. 6. Egg III

Larva I

Three specimens of this larva are present in the collection. The larva is transparent and without chromatophores. It is 7·7 mm. long from the snout to the caudal end with a maximum height of 1·2 mm. including the fin-fold. The head is short and about 1 mm. long (Fig. 7). The snout is very short and blunt (Fig. 8). The gape of the mouth extends to the anterior third of the eye. The lower jaw is slightly longer than the upper, and both the jaws are provided with four pairs of slender, tapering and anteriorly directed teeth which originate from the jaw cartilages (Fig. 9). The first pair of teeth in the upper jaw, 0·5 mm. in length, is the best developed. The succeeding pairs are relatively short, but the last one is very small. The teeth of the lower jaw are more or less of the same size. The myotomes are well formed and distinct in the anterior region, while those of the posterior region, especially the postanal ones, are narrow, closely arranged and consequently not clearly distinguished. About 146 myotomes could be made out in the larva. The alimentary canal is very long and straight and the vent opens to the exterior at a level with the 84th myotome. The anterior third of the alimentary canal is narrow and this region is abruptly followed by the remaining wider region. No traces of yolk are seen in the larva. The larval fin is continuous from the nape to the anus. Rays have begun to form in the caudal region of the fin-fold. The pectoral fin is small, circular and without rays.

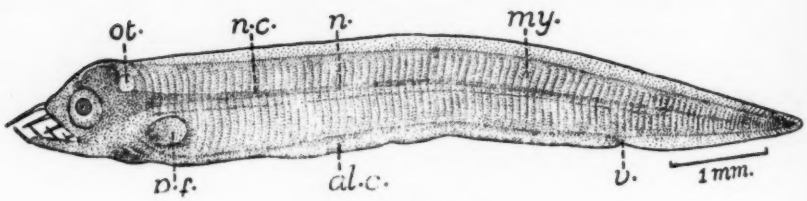

Fig. 7. Larva I

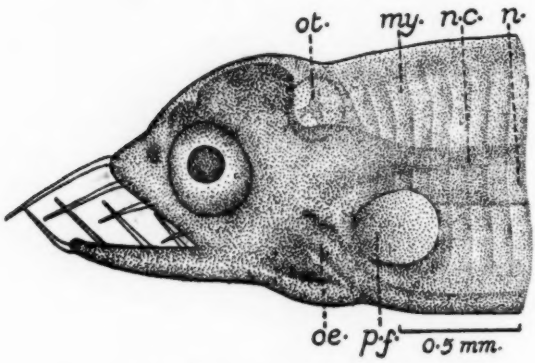

Fig. 8. Head region of Larva I

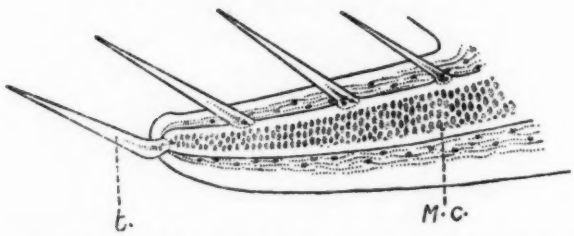

Fig. 9. Lower jaw of Larva I showing the origin of teeth from the Meckel's cartilage

Larva II

There are three specimens of this larva in the collection which could be easily distinguished from Larva I by the characteristic shape of the head and pigmentation (Fig. 10). The larva is transparent and 7 mm. in length from the snout to the caudal end and 1 mm. in height including the fin-fold. The head, 1 mm. long, is elongated with a long and sharply pointed snout (Fig. 11). The cleft of the mouth extends to the anterior border of the eye. The lower jaw is longer than the upper, and both carry four pairs of short, pointed and forwardly directed teeth. There are 78 myotomes clearly seen

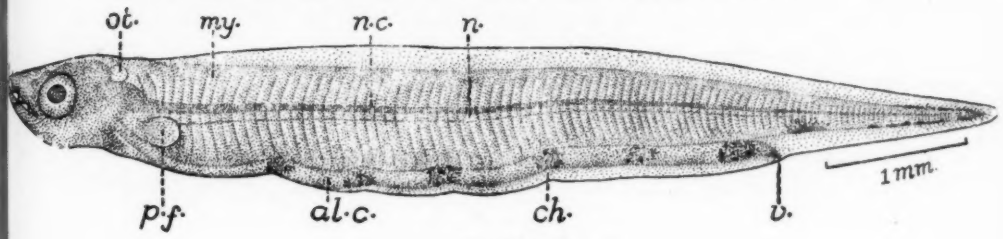

Fig. 10. Larva II

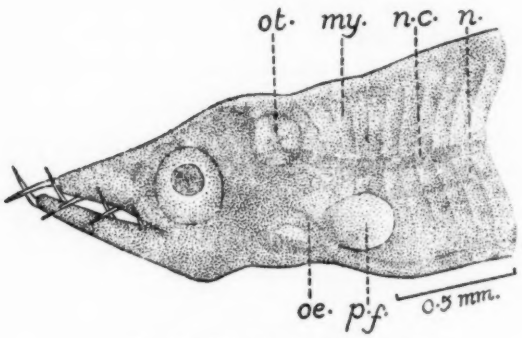

Fig. 11. Head region of Larva II

in the preanal region. The very compactly arranged postanal myotomes, numbering about 102, could be made out with some difficulty. The continuous fin-fold commences from the nape and extends beyond the vent up to two-thirds on the ventral side showing formation of fin-rays in the caudal region. The pectoral fin is small, rounded and without rays. The long alimentary canal is incurved at intervals having a festoon-like appearance. Six prominent groups of black ramifying chromatophores are present along the alimentary canal at the incurvations. A linear group of similar dense pigment cells is present behind the anus. Such chromatophores are irregularly found along the bases of the dorsal and ventral fin-folds of the caudal region.

Remarks

All the previous workers have experienced difficulty in determining the identity of the eel eggs studied by them and, with the exception of a few, the eggs have not been identified so far. This is also the case with regard to the many types of leptocephali recorded, the majority of which remains to be correlated with the adults. The identification of the eggs and larvæ described here is extremely difficult with our present meagre knowledge of the eels and leptocephali of the Indian waters. However, it may be men-

tioned in this connection that Nair (1948) recorded the leptocephali of *Congrellus anago*, *Uroconger lepturus*, *Muræsox cinereus* and *Muræna* sp. from the Gulf of Manaar.

The present study dealing with eggs in different stages of development including the very early stages would seem to indicate that Tholayiram Paar and neighbouring places afford favourable breeding grounds for Indian marine eels.

Acknowledgements

Our thanks are due to Dr. H. Srinivasa Rao for valuable suggestions and to Mr. K. Chidambaram for affording collection facilities in the Gulf of Manaar near Tuticorin and for furnishing the hydrological data.

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Key to Lettering

al.c.	alimentary canal	*oe.*	œsophagus
ch.	chromatophore	*o.g.*	oil globule
e.	embryo	*o.p.*	œsophageal pouch
f.f.	fin-fold	*ot.*	otocyst
h.	heart	*p.s.*	perivitelline space
h.r.	head region	*p.f.*	pectoral fin
M.c.	Meckel's cartilage	*t.*	tooth
my.	myotome	*v.*	vent
n.	notochord	*v.y.*	vacuolated yolk
n.c.	nerve cord		

ABRUPT SET-BACKS IN THE FISHERIES OF THE MALABAR AND KANARA COASTS AND "RED WATER" PHENOMENON AS THEIR PROBABLE CAUSE*

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Introduction

In the course of fish population studies along the West Coast in 1948, it was observed that there was an abrupt and a severe set-back in the fishery by about the middle of October within two weeks of the commencement of a bumper season. While we were investigating the probable cause of this dimunition, Mr. T. P. Bharatan, Assistant Director of Fisheries, Madras, drew our attention to the occurrence of the "red water" phenomenon at Hosdurg, one of the important fishing centres in the South Kanara District, where the fishing operations had practically come to a standstill. On further enquiry it was learnt that the phenomenon was widespread over a long stretch of the West Coast of India. The results of a study of the "red water" phenomenon at Hosdurg and of enquiries at other fishing centres along the Kanara and Malabar coasts, and a few general observations on the phenomenon of "red water" are recorded in this paper. A more detailed study of all aspects of the phenomenon could not be undertaken as, shortly after the investigation was started, the phenomenon declined and disappeared; however, the conclusion from the above studies and observations seems inescapable that the sudden diminution in the fisheries is due to the prolific and equally sudden multiplication of *Noctiluca miliaris* (Macartney), and their subsequent death and decomposition.

Previous Work

Though large-scale impoverishment of the fisheries such as was observed on the Malabar and Kanara coasts during October 1948 has not been recorded so far from other coasts, there are quite a number of records of localised instances of fish mortality. Hornell (1908) recorded a case of

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam.

severe mortality among sardine shoals in the sea near Mangalore in the middle of November. The area affected was a patch of the sea about fifteen miles long, one or two miles from the shore. The water in the affected area was greenish grey and turbid with unrecognisable organic debris in suspension. He (1917) also recorded numerous instances of mortality among marine animals in general and fish in particular near Calicut, and attributed the mortality to the occurrence of swarms of certain flagellates. This was corroborated by Jacob and Menon (1947). Chacko (1942) and Chidambaram and Unni (1942) recorded cases of mortality in the month of May among fishes and other marine animals near Krusadai Island in the Gulf of Mannar, due to the swarming of the blue-green alga—*Trichodesmium erythreum* and the subsequent fouling of water by their death and decomposition. Aiyar (1935) observed in June 1935 mortality in fish and marine animals near Madras caused by the occurrence of *Noctiluca* in exceptional abundance. He surmised that the mortality was due to the rapid utilisation by the organisms of available oxygen in sea water.

Nishikawa (1901) observed 'red tide' in Japanese waters due to the superabundance of the peridinian—*Gonyaulax polygramma* Stein, causing sometimes considerable destruction of Oysters. Gilchrist (1914) has shown that swarming of *Noctiluca* in South African seas has been responsible for severe mortality among the marine animals. Allen (1933–42) described in a series of papers the phenomenon of discoloured water in the La Jolla Bay, San Diego, California. In May 1933, the "red water" described was due to the preponderance of the Protozoan *Prorocentrum micans* Ehr. In September 1942 the "red water" observed was caused by the conspicuous abundance of *Gonyaulax polygramma* Stein. Allen has not, however, noticed mortality among marine animals as a result of the occurrence of these organisms in such enormous numbers as to discolour the waters. Davis (1948) has recorded a remarkable case of fish mortality in the Gulf of Mexico off the Florida Keys occurring sporadically over a period of nine months from November 1946 to July 1947 due to the superabundance in the sea of a new species of Gymnodinium—*Gymnodinium brevis* Davis, giving the sea surface a yellowish green colour. In a recent paper Brongersma (1947) has expressed the opinion that the fish mortality is caused by noxiousness of the red water of dinoflagellates; the occurrence of the red water in its turn being the result of the presence of upwelling water containing high concentration of nutrients. Marchand (1928) contends that the red water occurrences common in the South African coasts are revealed to be due to masses of *Noctiluca* and attributes the fish mortality to the decay of these masses of *Noctiluca* and diatoms polluting the water.

"Red Water" at Hosdurg

Investigations on the red water were carried out at Hosdurg, during the last week of October 1948. During the period of occurrence of the phenomenon, the south-west monsoon had ceased and the north-east monsoon had not yet started, and there was bright sunshine. The sea appeared to be comparatively calm and the water presented the appearance of a thick jelly-like slimy mass and contained decaying organic matter. The colour of the slime was dark grey due to the mixing up of the organic mass with the fine bottom clay. The fishermen of the locality found it extremely difficult to operate the nets. Cast nets would not sink, as they would do normally due to the thick slimy water, if operated, the fishermen could not drag the nets, on account of the weight of slime clogging the meshes; even the boats could not be steered properly through the water. Near Hosdurg, the sea bottom consists of a thick layer of fine brownish black clay. The bottom is often stirred by currents, and the clay particles remain suspended in water for fairly long periods.

A few horizontal plankton hauls were taken with considerable difficulty from the surface and at depths of four and fourteen fathoms. The plankton samples were fixed in five per cent. formalin for further detailed study. The surface plankton was very rich quantitatively and consisted almost exclusively of swarms of *Noctiluca miliaris* with a few other planktonic elements, such as lamellibranch and polychæte larvæ, copepods such as *Oithona* and *Euterpina*, diatoms and protozoans such as *Dinophysis*, *Coscinodiscus* and *Gymnodinium*. It may be stated that the minority elements constituted less than five per cent. of the total volume. When the plankton sample was allowed to stand for a while, the *Noctiluca*, many of them in active stages of fission, formed a thick layer of pink mass at the surface.

The plankton of the horizontal haul at a depth of four fathoms in the sixteen fathom area was dirty grey in colour, and consisted of a mass of mostly dead and putrefying *Noctiluca*, in different stages of disintegration. Most of the *Noctiluca* were shrunk or burst, but the flagellæ and cell walls were discernible in several individuals.

The plankton from fourteen fathoms in the sixteen fathom area was as slimy as that from the four fathom collection except that the slime was more profuse and mixed with black mud, a few fragments of *Noctiluca* and diatoms (*Coscinodiscus* and *Pleurosigma*), and a large number of foraminiferan shells.

An interesting feature of the phenomenon at Hosdurg was the occurrence of a few scattered zones of clear water, each about a furlong square in area,

which could be marked out distinctly from the affected regions. The slime-free parts of the sea, which may be appropriately termed " pockets " of normal plankton, rich and varied, contained a plankton fauna with the copepod element predominating. The following organisms are arranged in their order of abundance:—

Zooplankton	Phytoplankton
Oithona plumifera	*Coscinodiscus gigas* var. *prætexta*
Oithona rigida	*Coscinodiscus concinnus*
Acartia erythræa	*Pleurosigma angulatum* var. *strigosa*
Pseudodiaptomus sp.	*Rhizosolenia alata* f. *indica*
Corycæus furcifer	*Rhizosolenia robusta*
Euterpina acutifrons	*Ceratium massiliense*
Temora turbinata	*Ceratium furca*
Evadne sp.	*Ceratium fusus*
Polychæte larvæ (Spionids)	*Dinophysis miles*
Peneid zoea	*Dinophysis caudata*
Ostrea post larva	*Gymnodinium marinum*
Mytilus post larva	.. ..
Noctiluca miliaris	.. ..
Portunid zoea	.. ..
Carangid eggs	.. ..
Fish larvæ	.. ..

The *Noctiluca* in such areas was, however, conspicuously low in number though in excellent living condition. Under the binocular microscope, copepods could be observed clinging by their anterior extremities round bits of slime. It is difficult to say whether the segregation of zooplanktonic elements, particularly the copepods, in exceptionally large numbers in those " pockets " was due to their avoidance of the " red water " area and the consequent ingression into the clearer waters or to any physico-chemical factor prevailing in that part of the sea at the time.

The salinity and specific gravity of sea water in the affected area at Hosdurg on 23–10–1948 were 35·45‰ and 1·024 respectively. Other hydrological data such as oxygen, ammonia and nutrient salts could not be collected for lack of facilities. The study of the phenomenon could not be spread over a longer period and at other places as the phenomenon was already on the decline at the time of our investigation at Hosdurg.

The nekton of the area was very poor. A few dead fishes were found floating on the surface of water emitting a foul smell. Close to the shore a number of birds were found hovering over the surface of water picking

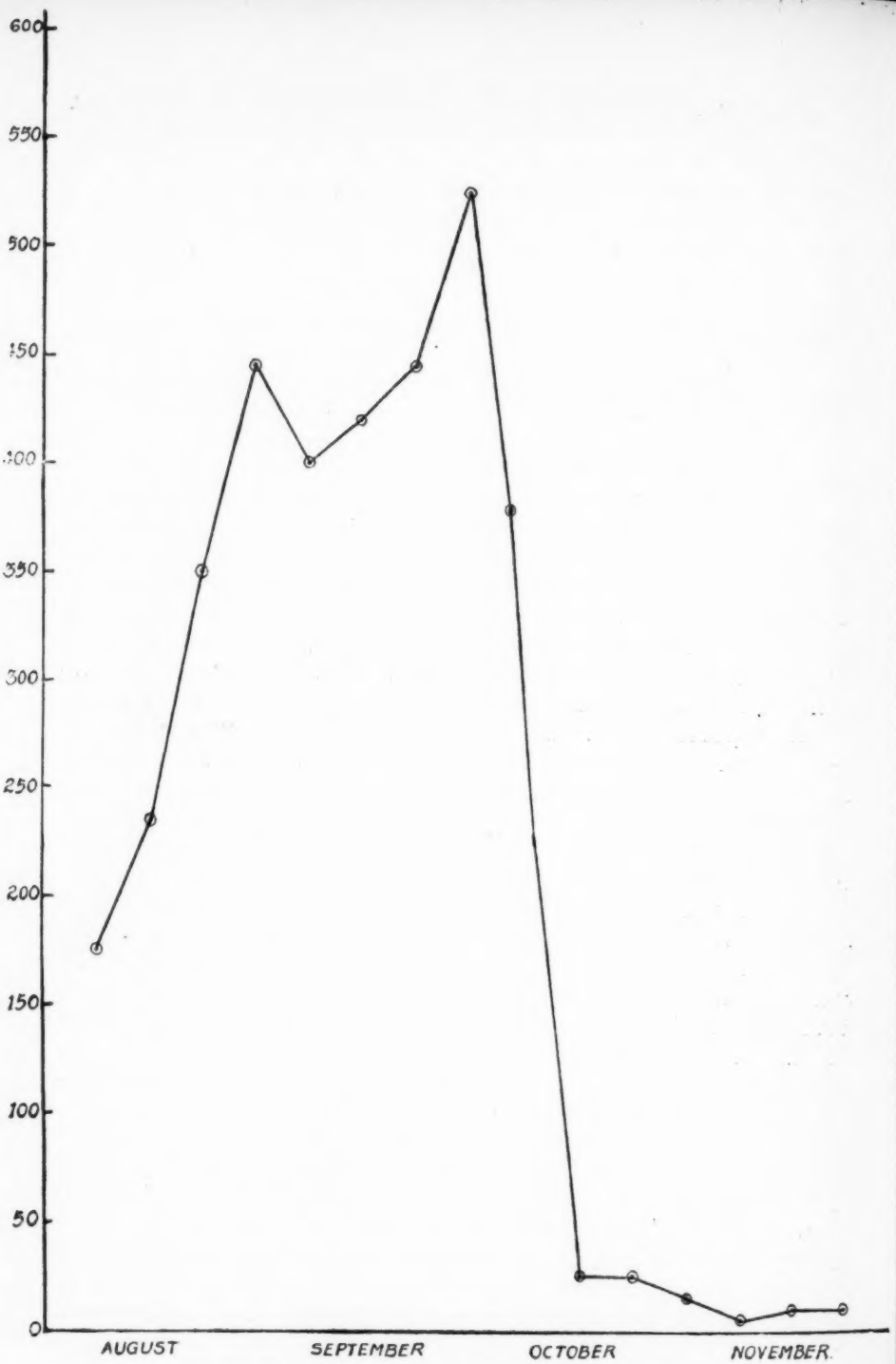
up the dead fish. Repeated netting brought no live fish from the area. The following species of dead fish were, however, entangled in the nets:—

Arius thallasinus	*Synagris japonicus*
Cynoglossus semifaciatus	*Caranx kalla*
Leiognathus bindus	*Carcharias* sp.

Examination of the stomach contents of the dead fish revealed the presence of a small quantity of the slimy debris found in the water. The gills and gill-rakers of all the fish examined were clogged with thick slime present in the sea water.

Widespread Occurrence of the "Red Water" Phenomenon

Enquiry showed that the phenomenon was widespread over an extensive stretch of coastal waters in the Malabar and South Kanara Districts. Pink patches at the surface of the sea first, and later thick slime in water were the initial indications of the set-back in the fishery during the period of its occurrence. At our request, the Assistant Director of Fisheries (Coast) furnished from forty-two Fish Curing Yards in the two districts, detailed information relating to the period and extent of the occurrence of the phenomenon, to the manner in which fishing was affected and to the occurrence of such a phenomenon in the previous years. This information and the results of our personal enquiries at a number of fishing centres, revealed to us that the phenomenon observed at Hosdurg, occurred on a stretch of coast about one-hundred and seventy miles long from about three miles north of Calicut in Malabar to a few miles north of Gangoli in South Kanara District, that it has not been known to occur at any time in the past, that difficulty in fishing operations was uniformly felt throughout the area of its occurrence, that fishes had practically avoided the affected areas and that it occurred for about ten to twenty days during the later half of October over a distance of about four to twenty miles from the shore. A consolidated report received from the Assistant Director of Fisheries (Coast) states: "Such a phenomenon was not observed anywhere on this coast within the memory of the oldest of the present generation. Even the very old fishermen say that they never saw such a phenomenon in the sea and that they have never heard their elders saying about it. Wherever it was seen the fishing became impossible and there were no catches during the period of its occurrence and some days after. The different kinds of nets used in the coast could not be cast as they would not sink in the sea with the use of ordinary weights and even if they sank, the fishermen found it difficult to pull them up as the slimy substance was sticking to the nets and the water would not pass out through the meshes. There are instances when the nets which



GRAPH I. *Noctiluca* Peak on the Calicut Coast, 1948
(Numbers denote *Noctiluca* in 1 c.c. of standardised plankton)

were cast in this water got torn when they were pulled up. Even hooks and lines could not be operated successfully as they did not go sufficiently deep in the sea and the sharks were not able to see the bait and swallow it. The lines got thick by the slimy substance sticking on to them. Fishing was comparatively a failure throughout the period of the appearance of the substance in the sea. The substance was reported to be visible with a thick layer of muddy water with purple colour and a peculiar smell."

Abundance of *Noctiluca* in the Sea near West Hill

It is significant that just prior to and during the occurrence of the phenomenon elsewhere on the coast, there was a marked abundance of the *Noctiluca* population in the sea near West Hill. From the records of the quantitative estimation of the triweekly plankton collections maintained at this laboratory, it is seen that *Noctiluca* made its first appearance in the year in the last week of May and continued to be present in normal numbers in the plankton during June and July. In August and September, its incidence gradually increased until in the latter half of September it attained a peak constituting the main bulk of the plankton. It was still dominant upto about the middle of October when there was an abrupt decline in its numbers. They practically disappeared from the plankton by the middle of November. The accompanying graph shows the relative abundance and scarcity of *Noctiluca* in the plankton during the period from August to November 1948.

During the peak period pink patches of *Noctiluca* were very commonly seen at the surface of sea water and were even washed ashore in large quantities due to tidal action. Examination of samples of sea water brought to the laboratory showed that considerable numbers of *Noctiluca* were dead and settling down to the bottom of the glass trough while the live ones formed a thick layer at the surface; and after two days it was observed that decomposition of the dead *Noctiluca* had set in and a large number of marine ciliates and flagellates had developed.

On the morning of 30th September an interesting case of shoaling of the Malabar sole—*Cynoglossus semifasciatus*—was observed. As there was bright sunlight and the sea was very calm we were able to see shoaling of large masses of the sole very clearly just below the surface of water. In spite of their active movements the fish could be netted easily. A single haul of a cast net brought in about 9,000 fish weighing 162 pounds. The fishermen were able to draw the net with the fish to the boat with very great difficulty. Further, a signal was given to a boat seine (Paithu vala) party who caught in a single operation of the net two complete boat loads of the fish. It is possible that the fouling of the bottom layers of water by the

dead *Noctiluca* was responsible for the occurrence of the habitually bottom fish at the surface. It was stated that at Hosdurg also the pink patches preceded the increase of *Noctiluca* population which formed thick slimy masses in suspension.

The Effect of "Red Water" on the Fisheries

The immediate effect of the occurrence of the "red water" or slimy water phenomenon was a steep fall in the fish catches over a long stretch of the coastal waters. It is clear from our own observations and enquiries that the bulk of commercial fishes—particularly the shoaling species, the chief among them being the mackerels and allied forms, sardines and anchovies—did not appear in such waters. There was, however, no severe mortality of fishes. It would appear that the physicochemical conditions of the waters thus affected were in some way unfavourable for live fish that they completely avoided such areas. Though the fishing season was favourable in the first half of October it deteriorated during the latter half of the month when the phenomenon occurred. Table I, which gives the total quantities of fish landed in October from 1944 to 1948 for the area covered by thirty-one Fish Curing Yards from Calicut to Gangoli, shows the extent of decline of fish-landings in October 1948 as a result of the widespread occurrence of the "red water" phenomenon. It may be added that the quantity of fish landed in October 1948 pertains only to the first half of the month, there being practically no fishing during the second half.

Table I

Total fish landings from Calicut to Gangoli

October 1944	..	5,14,860	maunds*
October 1945	..	7,87,980	,,
October 1946	..	4,55,084	,,
October 1947	..	5,59,828	,,
October 1948	..	2,42,970	,,

* 1 Maund = 80 lbs.

Plankton records show clearly that during the occurrence of the "red water" phenomenon there has been considerable destruction, not only of fish eggs and larvæ but also of plankton in general (diatoms, copepods and cladocerans) which forms the food of fishes. The repopulation of the area by these forms which would attract fishes to the coastal waters was observed to be slow. That the avoidance by fish of the "red water" zone was not merely due to the physical obstruction caused by the slimy masses of *Noctiluca* but also to the high toxicity of the water, was demonstrated by

the following simple experiment. A few live fish were kept in the filtered "red water" in glass troughs with separate controls containing sea water taken from an unaffected area. While the fish in the controls were perfectly normal throughout, those in the filtered water from the affected area, though normal at the beginning, showed signs of the toxic effects of the filtrant in two hours and died thereafter.

Discussion

It appears clear from the foregoing observations that the severe set-back in the fishery over a long stretch of coastal waters in the Malabar and South Kanara Districts during October 1948 was mainly due to the putrefaction of a superabundant *Noctiluca* population, giving rise to thick masses of slimy substance occurring in a state of suspension in the coastal waters. The most characteristic feature of the phenomenon was the extensive slimy masses of dead and decaying *Noctiluca* mixed partly with the fine clay of the mud banks characteristic of the area. It was therefore, considered appropriate to use the term "slimy water" for this phenomenon. But we have, however, preferred to retain the term "red water" since it has already been used by previous workers (Gilchrist, 1914; Marchand, 1928; Nishikawa, 1901) to indicate the abundance of *Noctiluca* in other seas. It must, however, be admitted that even in the present instance, to begin with, large pink patches of *Noctiluca* were noticed on the surface waters at several places in the coast, which soon gave place to the slimy masses.

It may be pointed out that the term "red water" has been used vaguely in the past. As has been observed under the chapter "Previous Work" this term has been applied to denote the superabundance of several forms such as dinoflagellates, euglenoids and peridinians. The hue presented by these forms varies with the individual organism concerned, and further with the intensity of their populations. In an instance such as the one during the euglenoid domination, the colour imparted to the sea water is definitely not red, as has been noticed by us on certain occasions. Hornell (1923) admits that the colour of this water is "amber brown" though he uses the term "red water". We agree with Jacob and Menon (1948) that the colour of the water during euglenoid abundance is brownish resembling hay decoction. Further the colour of the dense patches of *Noctiluca* is also not red in the strict sense but only pink. It is therefore necessary to define precisely the term "red water" and use it appropriately.

The researches of Gilchrist (1914) and Marchand (1928) have shown clearly that in African seas, thick masses of decaying *Noctiluca* bring about pollution of water resulting sometimes in mass mortality of fish and other

marine animals. The only record of mortality of fish in Indian waters due to the abundance of *Noctiluca* is by Aiyar (1935). Hornell (1917), however, asserts that *Noctiluca* is not an active agent in causing fish mortality. He states "I have seen it in great profusion colouring considerable areas bright pinkish red both off Cannanore and Palk Bay and in neither locality did I find any associated with fish mortality nor would any of the fishermen accuse it of evil influence; they agreed in declaring it to be innocuous". On the other hand we are inclined to think that the only case of severe mortality of fish recorded by Hornell (1910) off Mangalore was due to the abundance and subsequent death and decay of *Noctiluca* as his statement "a haul of plankton where dead sardines were plentiful almost entirely consisted of obscure organic unrecognisable debris, so fine that the meshes of the townets became clogged almost immediately, rendering it difficult to collect a satisfactory sample" agrees very well with what has been observed by us during the occurrence of "red water" in October 1948. It is true that mere abundance of *Noctiluca* unaccompanied by their death and decomposition is harmless to the fishery. The contention of Hornell that abundance of *Noctiluca* is innocuous to fish life while the dominance of euglenoids is the prime cause of mortality of marine animals, especially fishes, along this coast, appears to us to be untenable. Our laboratory observations show that marine euglenoids and ciliates develop in large numbers in glass troughs in which decaying *Noctiluca* are kept, indicating that the latter constitutes a good culture medium for these organisms. The same sequence seems to occur in nature as observed by us on the 29th August 1949 when dense populations of euglenoids mixed with a small number of ciliates made their appearance in the shore waters near West Hill. A bottom haul of plankton taken at 15 meter depth revealed the presence of a thick layer of putrefying *Noctiluca*. From the plankton records at our laboratory it was seen that for a week prior to this date, there was an abundance of *Noctiluca* in this part of the sea. At the same time it was observed that the fish catches for this date was very poor. There was, however, no fish mortality. In our opinion the avoidance of this part of the sea by the fishes is to be attributed to the toxicity and the physical obstruction caused by the putrefying *Noctiluca* rather than to the abundance of the euglenoids. Euglenoids constitute only a link in this chain of succession and cannot be regarded as the primary cause of the set-back in the fishery or the fish mortality.

As stated above the occurrence of *Noctiluca* "red water" affects fish life in every way. The water rendered slimy by the decaying *Noctiluca*, causes mechanical obstruction to the movement of fish while its foulness is toxic to fish life. The fishes either die or pass out of such waters. There

is also considerable destruction of fish food, fish eggs and larvæ. It appears to us that, the avoidance of such waters by the majority of the fish population is of greater consequence to the fishery production rather than the mortality of a few fishes. It has been observed on occasions that fish, which cannot immediately retreat from the "red water" zone, especially when the increase of *Noctiluca* is sudden, actually die. Detailed enquiries made at different fishing centres along Malabar and South Kanara coasts during the occurrence of red water in 1948, have shown that there was not a single instance of mass mortality of fishes anywhere; but on the other hand, the impoverishment of the fishery was noticed throughout the area. Generally, the shoaling species, which form the bulk of commercial fishes, shift from foul water areas to more favourable grounds in the neighbourhood. In the West Hill sea, the shifting of the fishery has been observed not only during periods of excessive production of *Noctiluca*, but also on days when dense populations of euglenoids, *Nitzchia* and *Oscillatoria* make their appearance. On such days the sea near West Hill which is normally a productive area presents a deserted appearance as the fishermen do not find it profitable to fish there. Beyond the affected area, however, the fishery appears to be good, as has been observed by us in the course of fish population studies. The local fishermen seem to know the shifting nature of the fishery when the "red water" is on, and the places where they can get a good haul.

It is suggested that the frequent occurrence of foul water, whether due to *Noctiluca* or other forms of Protozoa, diatoms, etc., with its attendant toxicity and destruction of fish food may possibly be one of the factors responsible for the scarcity of the oil sardines, which once contributed to the bulk of the commercial fisheries along this coast.

From previous records as well as the present investigation, it is evident that there is a definite periodicity (September to November) in the occurrence of foul water, along this coast in varying intensities, causing adverse effects on fishery in general. There are many gaps in our knowledge of this phenomenon. The causes of the sudden outburst and multiplication of organisms which result in foul water are at present obscure. The severe turbulence of the sea during the south-west monsoon period on the Malabar and South Kanara coasts possibly combined with upwelling in the offshore region having impact on inshore waters, the stirring up of the fine bottom sediments characteristic of this part of the coast and the sea currents may be severally responsible for the phenomenon. Sewell (1932) has adduced evidence to show that "the last part of the Antarctic Bottom Drift, after passing across the South of Ceylon is deflected by the Maldive ridge to the north and flows

northwards into the Laccadive sea especially along its east margin". The physicochemical factors responsible for the immense production of *Noctiluca* and other forms, the different organisms which cause foul water, the causes of their death and decay, the maximum density in their population for bringing about the adverse effect and the precise effect on fish and other marine animals are some of the problems which require detailed elucidation and investigation.

Acknowledgements

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THE MECHANISM OF TONUS IN UNSTRIATED MUSCLE

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Unstriated muscle exhibits two kinds of tone (Singh and Singh, 1946; 1947; 1948 *a*, *b*; 1949 *a*, *b*; Singh, 1949); they have been termed as lactic and alactic tones respectively. There appear to be separate contractile mechanisms for these tones. In the present research, further evidence is presented about the existence of these two kinds of tonic contractions and their mechanism elucidated.

Experimental methods were as described previously; stomach muscle from *Rana tigrina* and unstriated muscle from dog's stomach have been used.

Results

Tonus in frog's stomach.—The cardiac and pyloric halves behave differently as regards their tonic properties. The muscle from the pyloric half, when put under tension of about 30–40 g., can maintain this tension for hours. The tension is not due to visco-elastic properties, as when relaxed, the muscle can lengthen more without exciting any tension. The stomach is taken out immediately after the animal is killed with a blow on the head, so that the tonic contraction described above represents normal tone of the muscle.

The above tonic contraction has got all the properties of alactic tone described previously. It is resistant to asphyxia or cyanide and adrenaline (1 in 10^7–10^5) does not produce the slightest inhibition, though it may produce a contraction. It can be slightly diminished by glucose or alternating current.

The cardiac portion, especially from near the cardio-œsophageal sphincter exhibits all the properties of lactic tone. It is sensitive to asphyxia or cyanide. It is very sensitive to adrenaline; 1 in 10^7 may almost completely abolish it. Muscle from intermediate portions may exhibit intermediate properties.

The tension due to tonic contraction can be destroyed by forcible stretching without affecting the twitch contraction produced by alternating current (Fig. 1), showing that the two are due to separate contractile elements. The

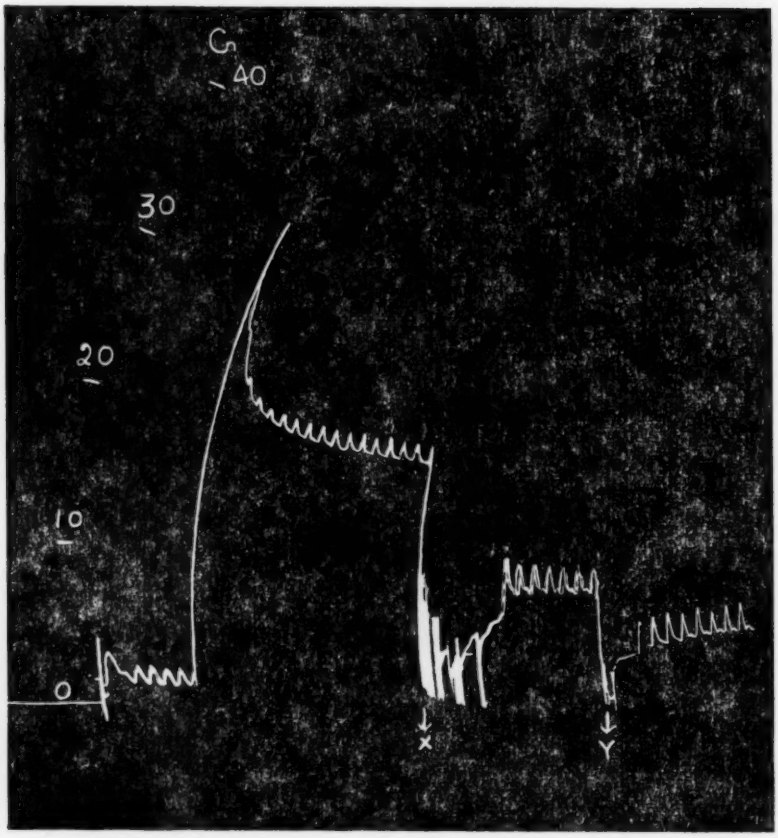

FIG. 1. *Frog stomach muscle from pyloric region.*—The muscle is being stimulated with alternating current 12 volts / 10 sec. per minute. The muscle is put under tension. The tension is then destroyed by forcible stretching at *X* and *Y*. Note that the twitch contractions are uneffected.

properties of the tonic contraction of the pyloric end of the stomach therefore exactly resemble those of the asphyxial contraction, and these experiments therefore confirm the views mentioned previously regarding the nature of alactic tone.

In dog's stomach there are similar difference between the properties of the muscles from cardiac and pyloric ends, but they are not so marked as in the frog's stomach. The variability of the tone of the stomach probably accounts for the variable action of sympathetic and vagus nerves. Sympathetic stimulation would result in contraction or there would be no response

if the muscle is in alactic tone; it wou.d result in inhibition if in lactic tone. Similarly vagus stimulation would result in contraction of a relaxed muscle, and if already contracted it would relax the muscle, as lactic tone may be neutralised as result of stimulation. The achalasia of some sphincters in the body is probably due to change in the nature of the tone; a change of lactic to alactic tone would cause a failure of relaxation on the arrival of an inhibitory impulse. Vascular hypertension may also be due to change in the nature of tone of the blood vessels.

The reaction to stretch.—Distilled water causes an unloaded frog's muscle to elongate activity. It has been found that if the pyloric half of the stomach muscle is put under tension of about 30 to 40 g. and then immersed in distilled water, this elongation is prevented. The greater the tension, the greater its antagonistic effect on elongation; this is shown by using an isotonic lever. Thus the muscle may relax with small loads (5 g.) but not with larger loads (20 g.). The muscle may swell by more than 100 p.c. and yet not elongate, showing that the swelling is not the cause of elongation.

The pyloric and cardiac halves of the frog's stomach muscle differ remarkably in their reaction to stretch and distilled water. The pyloric portion does not relax at all when put under tension and then immersed in distilled water (Fig. 2), whilst the cardiac portion begins to relax immediately. The relaxation must be distinguished from the linear decrease of tension due to the viscoelastic properties of the muscle. The muscle from the cardio-œsophageal sphincter relaxes most. When reimmersed in saline or isotonic sodium chloride solution, the pyloric portion relaxes and the cardiac portion contracts, though after 24 hours immersion it may relax instead of contract. These reactions are produced by living as well as dead muscle. They may be produced after half to one hour's immersion in distilled water, a procedure which does not kill the muscle or they may be produced after 24 hours' immersion, a procedure which kills the muscle.

These experiments with distilled water and the behaviour of dead muscle suggest that in unstriated muscle there are two kinds of proteins responsible for the two kinds of tone respectively.

The dog's stomach muscle, whether from the cardiac or the pyloric portion contracts when put under tension and immersed in distilled water, so that it predominantly contains the protein responsible for alactic tone. The gut muscle behaves oppositely, so that it predominantly contains the second kind of protein. Various muscles probably contain various mixtures of these proteins and thus differ in behaviour.

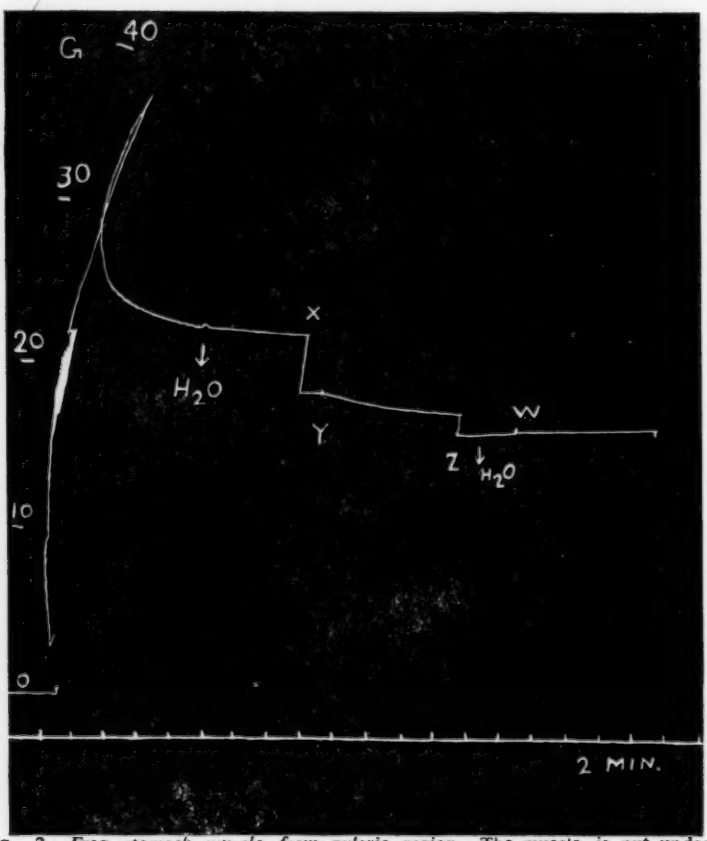

FIG. 2. *Frog stomach muscle from pyloric region.*—The muscle is put under tension and then immersed in distilled water. Drum stopped at *X* for 24 hours. Isotonic NaCl added at *Y*. Drum stopped at *Z* for an hour, and distilled water add at *W*. The muscle begins to contract again. Tension decreases due to its viscoelastic properties.

The muscle elongates in isotonic potassium chloride and other solutions (Singh and Singh, 1949 *c*) and stretching antagonises this elongation.

The behaviour of living muscle.—The decrease of tension produced in muscle immersed in distilled water by increasing the osmotic pressure can also be produced in living muscle immersed in saline. Thus if the muscle is put under tension in saline, then by doubling the osmotic pressure of the saline by adding sodium chloride produces similar reduction of tone, showing that the proteins of living muscle behave like those of the dead muscle immersed in distilled water. The muscle therefore contains a mechanism whereby it is able to maintain tension without expenditure of energy. This

is also proved by the fact that the oxygen consumption of the muscle (Rao and Singh, 1940) and lactic acid production (Bharadwaj and Singh, 1950) diminishes if the muscle is stretched. The diminution of metabolism is due to the fact that the alactic mechanism takes over from the lactic one.

Stretching also produces a contraction which requires energy so that it may stimulate the mechanism of lactic tone.

Stretching, therefore, induces a change in the contractile protein of the muscle which increases the tendency to contraction and diminishes that to inhibition (Fig. 3). It is remarkable that asphyxia also produces similar effects, inhibition being converted into contraction (Singh and Singh, 1949 *d*).

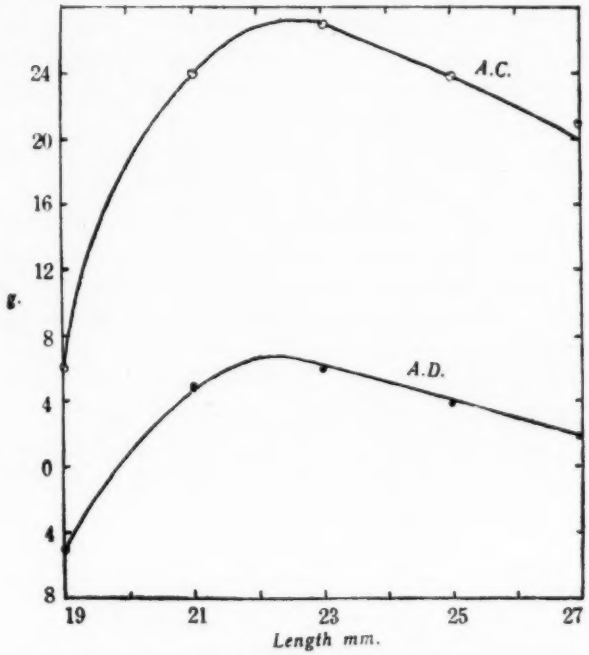

FIG. 3. *Frog stomach muscle.*—Effect of initial length on contraction produced by alternating current (A.C.) and inhibition produced by adrenaline, 1 in 10^8 (A.D.). With small in initial length there is inhibition, and as the length is increased inhibition is converted into contraction.

DISCUSSION

Previously it has been described how unstriated muscle reacts to diminished oxygen supply. These experiments show that the muscle acts similarly if the demand is excessive. When increased demand is made on the

muscle with normal oxygen supply or normal demand with diminished oxygen supply, it puts into action a contractile mechanism which does not require energy. The muscle thereby is enabled to maintain tension without extra expenditure of energy. As a matter of fact the energy expenditure is diminished, because the alactic mechanism takes over from the lactic one. To understand the properties and reactions of unstriated muscle, the nature of tone has to be taken into account.

Summary

1. Frog's and dog's stomach show the predominance of alactic tone in the pyloric regeion and lactic tone in the cardiac region.

2. The tone in the pyloric region has the properties of the asphyxial contraction.

3. The tension due to tonic contraction of the pyloric muscle can be destroyed without affecting the twitch contraction suggesting that these two are mediated by different contractile mechanisms.

4. Stretching of muscle antagonises elongation produced by distilled water and other substances.

5. The muscle from the pyloric and cardiac regions behave differently in distilled water. The former contracts and the latter relaxes. These reactions are produced both in living as well as dead muscles, so it is concluded that unstriated muscle contains two kinds of contractile proteins for lactic and alactic tones respectively.

6. Stretching increases the tendency to contraction and decreases that to inhibition. Asphyxia also produces similar effects.

7. When increased demand is made on unstriated muscle with normal oxygen supply or normal demand with diminished oxygen supply, it puts into action a contractile mechanism which does not require energy.

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